JOURNAL OF AGRICULTURAL RESEARCH

VOL. 35. WASHINGTON, D. C., SEPTEMBER 1, 1927 No. 5

THE UTILIZATION OF CARBOHYDRATES BY HONEYBEES[1]

By E. F. PHILLIPS

Professor of Apiculture, New York State College of Agriculture, formerly Senior Apiculturist in charge of Apiculture, Bureau of Entomology[1]

INTRODUCTION

In the study of the problem of wintering honeybees it was found that the character and composition of the food plays an important part in successful wintering. It was also suggested from certain findings in that study (*63*)[2] that not all carbohydrates included in the normal food of bees are utilized and that undigested carbohydrates contribute considerably to the accumulation of feces during the winter confinement to the hive. The presence of an undue amount of feces was found to cause greatly increased muscular activity in bees in winter confinement, since bees do not normally void their feces in the hive, but, if possible, retain them until an opportunity for flight occurs. This increased muscular activity results in the rapid death of individual bees and of the colony as a whole. An especially destructive result is the loss of vitality in those bees which still live. Any condition which causes an accumulation of feces is, therefore, highly undesirable, bringing about the condition known to beekeepers as dysentery.

The apparent difference in the availability of various carbohydrates as food suggested a study of this problem with chemically pure materials, and the work was carried further to include some carbohydrates which bees would not encounter in nature. Since so little is known of the utilization of certain rare carbohydrates, it has seemed worth while to include these to test their availability for an animal which can be controlled easily and for which small quantities of the rare and often expensive carbohydrates would be sufficient to obtain a definite result. In the case of the simple sugars, the question is whether they are capable of absorption and utilization; while for the more complex compounds the question is primarily whether the bees can supply the enzymes necessary for the first breaking down of the compound sugars into simple sugars which must occur before absorption.

It also seems desirable to investigate this subject because of certain peculiarities encountered in practical beekeeping. The darker honeys (except buckwheat honey, in which the dark color is presumably caused by tannin bodies) and honeydews, if given as food during the period of winter confinement, are the materials which cause dysentery.

[1] Received for publication May 17, 1927; issued October, 1927. The feeding experiments as well as some of the calculations herein recorded were made while the author was connected with the Bureau of Entomology. The work was completed and prepared for publication at the New York State College of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 427.

Feces may accumulate to the point where the bees are no longer able to retain them, resulting in spotting of the interior of the hive. The ease with which this condition is relieved if a flight permits the voiding of feces indicates that the presence (pressure?) of feces alone causes the abnormal behavior. The dark honeys and honeydews differ from superior honeys chiefly in their higher dextrin content, suggesting, but by no means proving, that the adult bees are unable to utilize dextrin as food. Earlier investigators have reported the presence of amylase in adult bees, as well as in honeys, and if this enzyme were actually present in the lumen of the alimentary tract, the enzyme capable of hydrolyzing dextrins (dextrases, assuming that there are several enzymes involved in the complete hydrolysis of starch), might also be expected to occur in the lumen of the alimentary tract. If this were the case, a higher percentage of dextrin could hardly be the cause of the great accumulation of unutilized material in the form of feces. The bearing of earlier work on the present problem will be discussed when the experiments on feeding starches and dextrins are detailed.

A short, nontechnical discussion of some of the results of the work herein recorded was published (*62*) by the author in 1924, to present to beekeepers some of the practical bearings of this problem. Bertholf in a related investigation (*10*) finds that honeybee larvae are able to utilize dextrins, lactose and galactose, which appears to be untrue for adult bees. The experiments herein recorded were undertaken in connection with the work done by Demuth [3] and the author on the behavior of bees in winter.

CARBOHYDRATES AND ENZYME SOURCES INVOLVED IN BEE DIGESTION

Before entering upon a discussion of the experimental work, it seems desirable to suggest complicating factors which may be involved in a study of the utilization of carbohydrate by the honeybee. This is done in order to show the reason for the simple technic here used and to suggest sources of errors which may have entered into the results obtained by others.

The usual method of determining the enzymes present in various organs is to make extracts of the tissues or of digestive juices in water, glycerin, or weak alcohol and to test the action of these extracts on known substances. This method has been used in investigations of the digestive processes of the honeybee. That these methods are open to some criticism will appear in later discussions, especially under the discussion of the utilization of starch by the bees. In making such extracts of the alimentary canal of the honeybee, it appears to be impossible completely to remove the included pollen, which is a prolific source of enzymes. It is also impossible to obtain only the digestive juices or the tissues producing them free of muscle and other tissues, which may introduce an error. It is the belief of the author that such sources of errors are responsible for some of the apparently incorrect results which have been obtained by other investigators.

It is possible to investigate the utilization of various carbohydrates by the honeybee by the methods used in the present instance,

[3] The author wishes to acknowledge the assistance given by George S. Demuth in the first three series of feedings, and by H. S. Paine, in charge of the carbohydrate laboratory, Bureau of Chemistry, for valuable help in planning some of the later series of feedings.

because of the ability of honeybees to live for considerable periods when taking no food other than chemically pure carbohydrates. During the larval period bees consume large quantities of protein, including pollen (*56*) which are rich in protein, during the latter part of larval life. During adult life worker bees consume large quantities of pollen [4] when engaged in the elaboration of larval food and the feeding of the queens. Otherwise during adult life the workers appear to ingest little or no pollen; and it is known that during winter when they are confined to the hive and when no brood is being reared, bees apparently need little or no pollen and can be carried through the winter (perhaps five months) successfully with no pollen in the hive.[5] Experiments such as are here described would be impossible with any species which requires a mixed diet at all times. To what extent worker bees under such circumstances are drawing on their protein reserve need not concern us at this time.

MICROORGANISMS IN THE ALIMENTARY CANAL

The problem of testing the utilization of various carbohydrates may not be one involving simply the production of enzymes by the bees themselves. A considerable number of species of microorganisms occur in the intestine, and attention must be given to such members of this flora as might modify the results. This is important in view of the belief sometimes expressed that certain carbohydrates are hydrolized by the action of enzymes produced not by the mammalian alimentary canal but by microorganisms occurring in it. An even more striking case occurs in termites, in which cellulose is hydrolized by intestinal protozoa. There is, of course, the possibility that some of the bacterial species in the bee intestine produce enzymes which are involved in the hydrolizing action in bee digestion.

In considering this question one is concerned chiefly with those microorganisms which are regularly found in the bee intestine and not so much with species which are rarely found or which are incidental visitors. From the work of Bruce White (*76*) it is possible to differentiate the organisms that are normally present, at least in Scotland where his work was done. In this group of bacteria (*Bacillus constellatus, B. rigidus, B. influenzoides apis,* and *B. tenusi apis*), all four grow on media containing glucose and three on maltose media. None were reported to grow on media containing other sugars or on milk. *B. rigidus* grows feebly on potato while *B. tenusi apis* grows well on this medium. White (*76, p. 76*) concludes: "The organisms are as a whole characterized by their predilection for glucose-containing media and the fermentation of this sugar with-

[4] There is a large literature on the composition of pollen. For summaries of this work, see Parker (*56*) and Paton (*57*). The fact that certain pollens cause hay fever in susceptible persons has led to extensive studies of pollen, and the literature on hay fever may profitably be consulted.

[5] That bees do not need to ingest any protein during winter may perhaps be explained by the storage of protein material in the so-called fat body (*42*). It was at one time believed by some American beekeepers that dysentery in winter is caused by the consumption of pollen, and to remedy this condition it was advised that all combs containing pollen be removed from the hive during winter. It is now known that pollen consumption does not explain dysentery, and that bees are able to live through the winter without pollen (*55*). For those unfamiliar with the methods of beekeeping, it may be stated that when the pollen-bearing combs were removed, it became necessary for the beekeeper to provide additional carbohydrate food, which was done by feeding sugar sirup. This reduced or entirely eliminated the honey containing indigestible ingredients, thus preventing dysentery; whereas the beneficial results were attributed to the removal of pollen, which had nothing to do with dysentery. This removal of pollen had an indirect bearing on this trouble, however, for without pollen no brood could be reared, even though the temperature conditions became favorable for that activity; and the large addition to the feces, which would have resulted had brood rearing been carried on, was therefore prevented.

out gas production." The only organisms in the intestinal flora, which were found to grow on media containing lactose or sucrose, were inconstant species.

There are also yeasts and other fungi present in the intestine of the worker bee, but these are not a large factor in the flora. It would appear that because of their relative scarcity none of these fungi need be considered as likely to complicate the problem considered in this paper. In addition to pollen grains and microorganisms, there are present in the intestine hairs from the outside of the bee and various foreign bodies. Most of these probably play no part in hydrolizing the carbohydrates previous to absorption, but the presence of included material, such as pollen grains, may materially modify certain experimental results on the presence of enzymes, as will be seen later in an examination of the work of Petersen on starch digestion.

VITAMINS IN THE DIET OF HONEYBEES

Feeding experiments such as those recorded herein cannot be discussed without raising the question of the vitamin requirements of bees, a subject on which little is so far known. Honey, the normal carbohydrate food of bees, has been studied for its vitamin content (*5*, *20*, *22*, *26*, *34*, *69*), and most workers find none. Pollen, on the contrary, has been tested only by Dutcher (*22*) who finds it effective in curing avian polyneuritis. It might be expected that pollen would contain at least vitamins A and B. The various microorganisms which occur in the alimentary tract of the adult bee may also be a source of vitamins, although none of them have been tested in this respect.

The question of the importance of vitamins to adult bees under normal conditions need not especially concern us in connection with the present problem. It is enough to know that under the conditions of the experiments and with the bees selected at random from the colonies, some of them survived for 30 days without obtaining food containing vitamins. Whether these bees were normal is not determined, and the only question at present discussed is whether or not they can utilize the carbohydrates given them, to which question they have given a definite answer. It is a fact of considerable importance and interest, however, that beekeepers consider it good beekeeping practice to give their bees a sirup of partially inverted cane sugar for the period of winter confinement, often extending over five months. Under the conditions within the hives during this time it seems that the bees survive and are in excellent condition for building up strong colonies in the spring on food free of vitamins. If honey does not contain vitamins, the bees, nevertheless, normally survive winters without vitamins in their food.

In spite of the number of workers who have tested honeys for vitamins, it is still perhaps safe to state that more work is needed. Honeys from various floral sources vary considerably in their chemical composition, flavors, and in many other distinguishable ways. It may therefore be that they vary in vitamin content. This is suggested by the marked variation in amber or yellow color, since vitamin A is associated with yellow carotin color at times. Carotin is one of the chief constituents of honey colors, and this suggests the desirability of conducting tests for vitamins with amber and dark honeys.

This is mentioned here with a realization that it doubtless has no bearing on the present problem. However, since the darker honeys, those containing more carotin, are known by beekeepers frequently to be the cause of dysentery, this in turn suggests that vitamins are unnecessary during the period of winter confinement to the hive, during which period brood is not normally reared.

ENZYMES IN HONEY

Considerable work (*2, 3, 4, 18, 23, 24, 25, 27, 30, 44, 45, 46, 47, 52, 66, 75, 81*) has been done on the presence of enzymes in honey, much of this work having been undertaken in an effort to find means for detecting adulteration. It is concluded that some of these enzymes have their origin in nectar (for example, catalase, according to Auzinger), and that others are produced by the bee and are added during the ripening process. This has a distinct bearing on the present problem, especially in view of the fact that several of the investigators of this subject report the presence of amylase in honey. This was reported first by Erlenmeyer and von Planta. Auzinger makes the definite statement, however, without any evidence to support it, that this enzyme is produced in the salivary glands. If this were the case, it would doubtless occur in the ventriculus of the bee and would serve for the hydrolysis of starch and dextrins (on the assumption that the enzyme present is the mixture of enzymes necessary for the complete hydrolysis of starch to glucose). In view of the fact that in these tests the pollen in honey is evidently not eliminated as a possible source of enzymes (Auzinger states definitely that the honeys were not filtered), and in view of the presence of amylase in pollens (*57*), it appears at least possible that the results reported are not fully trustworthy, in so far as these enzymes are assumed to be produced by the bees. That bees add invertase to nectar during the process of ripening is well known.

An enzyme to be functional in the processes of digestion of carbohydrates must be present in sufficient amount to act on the carbohydrates after they are ingested and must act so that the carbohydrate food, when there is but one present, may be hydrolysed fast enough to maintain life. This presumably means that no enzyme not produced by the bee itself will serve the purpose. To a considerable degree, therefore, the results obtained in the study of the enzymes in honey may be disregarded, at least in so far as the enzymes come from nectar. The detection of enzymes in honey by the use of glycerin and alcohol extracts should not be interpreted as meaning that the enzymes are produced by the salivary or digestive glands, as Auzinger seems to have done.

INVESTIGATIONS OF ENZYMES IN THE BEE

The physiology of the digestive processes in the honeybee has been investigated by Petersen (*59*), who reviews the essential physiological literature on this subject to the date of publication. The work which he did is quite unlike that herein recorded, and it is not necessary to discuss all his findings. On one point only is it necessary to examine his work in detail, and this will be done under the discussion of the utilization of starch. Biedermann (*11*) carefully reviews the literature on digestion of bees and other insects up to the time of publication.

While the present work was under way, there appeared an extensive paper by Pavlovsky and Zarin (*58*), which deals chiefly with the enzymes present in the various parts of the alimentary canal of the honeybee. The methods used by these authors were those usually employed in such work, namely, the preparation of enzyme extracts with glycerin or alcohol from the various organs to be studied. These authors record that they carefully washed out the included material (a precaution which Petersen does not record taking), which would presumably eliminate pollen as a source of enzymes to disturb their results. It is greatly to the credit of these investigators that they succeeded in getting out all the included material, for this must have been a difficult task. They report the presence of amylase (diastase) in the ventriculus. There are several places in the paper of Pavlovsky and Zarin in which their results are apparently stated incorrectly. For example, they state that they established the presence of lactase (*58, p. 519*) and later they state that they did not find lactase present (*58, p. 527*), while in the table summing up their results (*58, p. 543*) they record the presence of lactase in the stomach (ventriculus?). Sarin (*82, 83*) reports no lactase in the "stomach." Whether these errors were due to unfamiliarity with the language in which the paper appeared, which is not the native tongue of the authors, can not be learned. The paper reached the editor of the Journal in English and was not translated by him. The details wherein the results of these authors differ from those obtained in the present work will be mentioned under later headings in this paper.

CARBOHYDRATES AVAILABLE TO HONEYBEES

In order to understand the significance of the results obtained in the present work, it is desirable to review briefly the sources of the carbohydrates obtained in bees and the processes through which they pass in the formation of nectar within the plant, in the ripening of the nectar within the hive, and during digestion by the bee. Nectar, as obtained by the bees from floral and extra-floral nectaries, usually contains considerable sucrose, but there are small and varying amounts of glucose, levulose, and dextrins, together with the ash, water, coloring materials, and aromatic substances which are not considered in the present discussion. Some nectars probably contain no sucrose. The composition of various nectars has been discussed by various workers. (*13, 17, 19, 68, 78.*)

According to Bonnier the formation within the flower of a soluble food supply (sugar) as distinguished from starch is primarily that the flower may have a quickly available food reserve close at hand for the rapid development of the ovules after fertilization. It is only in certain plant species that an external secretion occurs, although the accumulation of sugar at the base of the flower occurs generally. The preliminary transformation of the insoluble starch (if the plant food supply is stored in that form) into soluble materials (usually sugars) is of interest here only in so far as the transformation from starch into sugars is sometimes incomplete, leaving a certain percentage of dextrins in the nectar. Since sucrose is not produced by hydrolysis of starch (*38*), the origin of this sugar, which in many cases is the chief constituent of nectar, is still somewhat in doubt. Since

sucrose and levulose may be produced directly by the action of chlorophyll, and as these are soluble materials, it is probable that the sucrose in nectar is produced directly by the chlorophyll and transported to the flower from the point of origin, and that it is not derived from insoluble storage foods. Invertase is generally found in plants and is doubtless involved in the formation of sucrose. Such a supposition would tend to explain the great variation in the composition of the secretion under varying external conditions, since in most plants sucrose is less abundant than is starch. The dextrin in nectar, however, is probably the result of the hydrolysis of starch, and the same may be true of the glucose. These points are of interest here only in so far as they suggest a reason for the increase in the percentage of dextrin under conditions when secretion of nectar is less rapid. It is well known to beekeepers that when secretion of nectar is slow, the honey from any given source is darker in color, and this is associated with an increase in the dextrin content of the nectar and consequently of the honey. The presence of dextrin in honey and honeydew honey indicates that it is not hydrolysed during the ripening process. There are also interesting variations in the color and abundance of nectar according to altitude, latitude, and other factors, some of which were pointed out years ago by Bonnier but which have become more clear with the great increase in beekeeping in the United States.

The production of nectar on the outside of the tissues of extra-floral nectaries is not a common occurrence at ordinary altitudes and latitudes. When such secretions occur the material exuded is as a rule higher in dextrin content than nectar from floral nectaries. Bonnier and Flahault (*14*) found that at high latitudes such external secretions are much more abundant than from the same plant species at points further south. A similar abundance of extra-floral secretion on the quaking aspen (*Populus tremuloides*) may be observed at high altitudes in the Rocky Mountains during the period of the growth of the leaves. Since extra-floral nectar secretion is greater at high altitudes and high latitudes, where the winter seasons are longer, it might be assumed that in such locations the quality of the food for the bees would be a serious problem. In fact, however, the food in such locations is usually of the best quality for winter use because of the abundant secretion from floral nectaries. It is well known that bees do not gather honeydews, either of plant or of insect origin, when there is abundant floral nectar.

Nectar or honeydew is collected in the honey stomach of the worker bee and brought to the hive. Here, after manipulation by the young bees inside the hive, it is deposited in cells of the comb, usually near the brood-rearing area. Only a small quantity is placed in each cell at first. By fanning their wings to create air currents, by raising the temperature within the hive especially near the brood nest, and perhaps by other activities of the bees as yet but slightly understood, the surplus water is evaporated. The remaining more concentrated solution (honey) is then brought together in the storage cells of the hive and is finally sealed over as fully ripened honey. During this ripening process a complete or almost complete inversion of the sucrose occurs, so that the final product, honey, contains but a small

amount of this sugar.[6] To this extent, therefore, the process preliminary to absorption occurs before the honey is actually taken by the bees as food. The enzyme necessary to the inversion of sucrose is present in all parts of the bodies of bees.

During a shortage of floral nectar bees may gather the excretions of aphids and other Hemiptera (insect honeydews) which they carry to the hive and manipulate just as they do nectar. There are few analyses of such material as it is gathered by the bees, but it is quite variable in water content, as can readily be seen when it occurs on leaves of trees. The ripening process is like that of floral nectar, but the final product (honeydew honey) differs from honey chiefly in the fact that the dextrin content is high. In the sample used in the feeding experiment recorded in this paper, the dextrin content was determined through the courtesy of the Bureau of Chemistry as 4.55 per cent. Another sample of honeydew honey received in 1916 by the Bureau of Entomology from Eagle Gorge, Wash., was found to contain 8.96 per cent dextrin. The usual dextrin content of normal honeys rarely exceeds 2 per cent, except in the most inferior honeys, and is less than 1 per cent in fine honey of the quality which beekeepers prefer to use for winter foods.

Other carbohydrates may also be found in honeydews or in honeydew honey after ripening. Unger (*74*) found mannite (a hexahydric alcohol) in considerable quantity on honeydew from *Juglans regia*, and Maquenne (*51*) found dulcite (a hexahydric alcohol) in honeydew from *Euonymus japonica*. As will be discussed later, melezitose (a trisaccharide) is found in certain honeydews. Because of the possibility of the presence of various carbohydrates in such compounds, it is desirable to determine the food value of many such substances to bees.

It may be well to point out that there is no suggestion that dextrins have any direct harmful effect on the bees. In the experiments carried out by Phillips and Demuth (*63*) on bees in a constant-temperature room (colony 3), the bees on honeydew honey stores lived but a short time. While they were undoubtedly nourished by the food provided them, the accumulation of feces stimulated them to great bodily activity and this activity in turn caused the temperature of the winter cluster to be raised. This should, however, not be interpreted as indicating that the accumulation of indigestible materials is in itself injurious; but the bees responded to this internal stimulus by increased muscular activity, which increased source of heat, raised the temperature of the cluster, and caused the bees to die of what the authors designated excessive heat production. No evidence was obtained that anything comparable to auto-intoxication occurs, although no examinations were made for microorganisms or other complicating factors which may have been present. The ease with which the condition known as dysentery may be relieved if the bees have opportunity for flight is against any such supposition.

It should also be pointed out that floral honey from any given species may differ greatly in the percentage of dextrin present. When honey is coming to the hive rapidly—that is when the flowers

[6] Not to exceed 8 per cent, according to the standard proclaimed Dec. 20, 1904, by the Secretary of Agriculture. There is considerable variation in the percentage of sucrose remaining in honeys from various floral sources, depending perhaps on the rapidity of the secretion and the consequent rapidity of the ripening process. Bonnier (*13*) states that mountain honeys (usually secreted rapidly) contain more sucrose than do those of lower elevations.

are secreting nectar freely—the resulting honey is lighter in color for the particular plant species than when the secretion is slow. This difference in color is associated with a difference in dextrin content, suggesting a more complete hydrolysis of insoluble carbohydrates to sugars within the plant or flower at the time when conditions are most favorable for nectar secretion.

It has been shown by numerous investigations that nectar secretion is more rapid, other things being equal, at high altitudes and latitudes. Honeys of the North are almost all lighter in color than those at lower latitudes. Since in northern localities bees are confined to the hives without flight for long periods in winter, often over five months, it is clear that the quality of food is of first importance in the successful wintering of bees in those regions. It is extremely fortunate for the development of practical beekeeping that the honeys of the North are light in color and free of dextrins, for bees are able to live in confinement of such honeys for the full period of the winter.

On the other hand, the presence of a higher percentage of dextrins in so many of the honeys of the middle and southern latitudes of the United States seems to explain the fact that the winter losses are usually as high in these places as in the extreme northern parts of the country. Most of the honeys which are produced in late summer and autumn are dark in color, such as the asters and goldenrod of the lower latitudes, and these are about as poor for wintering as any floral honeys available.

Bees also obtain carbohydrate material from the pollens which they consume, most of this material being glucose, levulose, and sucrose. Some pollens are found to contain starch grains (*72*) and this is probably the only source from which the bees would normally obtain starch as an article of diet under normal conditions. The complex carbohydrates which compose the pollen shell, celluloses, and pectinases, are believed not to be utilized by bees but to be cast out with the feces after the internal material has been removed by digestive processes. It is also believed that the contents of the pollen grains can not be digested unless the pollen coat has been broken before ingestion.

In the making of candy for the shipping of queen bees with accompanying workers, a finely divided cane sugar is mixed thoroughly with honey of good quality. Before the composition of confectioners' sugar was so carefully guarded by the administration of the Food and Drugs Act of 1906, it frequently happened that a beekeeper would purchase a lot of such sugar which contained a considerable amount of starch, and when this was used the death rate of queen bees and workers to which it was fed in confinement was found to be much higher than when a pure cane sugar was used. This indicated that the presence of the starch is detrimental to the bees, which would hardly be the case if they contained or produced amylase as has been claimed.

Since the sugar used in these cases is not known to have contained any material other than starch and ground crystals of cane sugar, the trouble must have come solely from the starch, as cane sugar is readily utilized by the bees as food.

It will be seen from the foregoing summary that the problem of the availability of various carbohydrates as food for honeybees is one of

practical importance. No attempt has been made in this work to study the processes of digestion of the various carbohydrates, nor to analyze the feces of the bees on various foods. Such work would be exceedingly difficult because of the activity of the bees and especially because of the relatively short life of bees under experimental conditions. By restricting the food to a single carbohydrate it is possible to tell whether the bees are nourished by it. The work recorded is therefore not chemical in nature, but does indirectly throw light on the chemical processes which must occur within the alimentary canal of the bee.

CONDITIONS OF THE EXPERIMENTS

Since bees kept in small wood and wire cloth cages in the laboratory are exceedingly active, due to the stimulus of light, the cages used in most of the experiments were kept in a cool, dark place in the cellar of the laboratory. Under these conditions the bees remained quiet, and because of this quietness doubtless lived much longer than they would in the light.[7] The cages used in the experiments were the same as those used by McIndoo (*49*) in his work on the olfactory sense of the honeybee. In the first two series of experiments about 100 honeybees were placed in each cage, while in the third series 200 bees were used. The third series of experiments were also modified by the fact that the bees were not placed in the cellar, but in a cool room in the laboratory where the light was not strong. The same methods were used in series 4 and 5 but no definite number of bees was used. Series 6 is discussed later.

In the first two series of experiments the bees used were chosen with great care by the following methods. The day previous to the beginning of the experiments the colony from which they were taken was moved carefully to a new stand in order that the field bees might return to their old location and that none of them would be included in the bees used in the feeding experiments. Then the bees used were hand picked to avoid very young bees which had not had a flight and which consequently have a considerable amount of feces in their rectums. By these means also the bees used were all about the same age. In the later experiments these precautions were not all taken, but the validity of the experiments were strengthened by the use of a larger number of bees. In all cases the field bees were removed by the moving of the colony, but in the later experiments a considerable number of bees were shaken into an empty box and were scooped up after being well mixed by shaking. In this way the handling of the bees was reduced and they were therefore subjected to less excitement and activity before the feeding experiments were begun. As previously stated, and as shown by the data presented, more bees were used in the later experiments, thus reducing the probable error of the averages.

The food and water provided were given in small vials inverted over the tops of the cages. These feeders were constantly moist but the contained solutions were held within the vials by atmospheric pressure. There was, of course, a slight loss of food from the fact

[7] That honeybees live longer when they have little work to do has long been known to beekeepers. It is quite outside the limits of the present discussion to give the evidence on this subject, but it is believed that bees are able to renew worn tissues to but a small degree, and that their term of life is shortened when they are called upon to perform muscular activity. The fact that during the larger part of their adult life bees use carbohydrates as food almost exclusively, perhaps explains this condition.

that some sugars crystallized out on the wire cloth and were not all used by the bees, but as it was not the primary purpose of the experiments to determine the exact amount of food consumed, this is considered an unessential detail. This crystallizing of the food was pronounced only in the case of melezitose.

The supply of food was renewed as necessary and counts were made of the dead bees daily, the dead bees being removed as counted. Except as noted, water was at all times available to all the bees, ordinary tap water being used for this purpose.

The honeybee is a cold-blooded animal (*65*), so that the temperature of the bees used in these experiments was the same as or only very slightly higher than that of the air surrounding them. The action of enzymes increases rapidly with a rise in temperature and is usually at its maximum at temperatures of 37° to 40° C. It may then be assumed that under the conditions of the experiments the enzyme action was reduced below that which would occur within the hive at a temperature of about 35° C. Since bees are able to live for long periods in winter at temperatures varying between 14° and 20° C. without injury, it is safe to assume that the temperatures encountered in these experiments were in no way preventive of enzyme actions of an essential character.

FIRST SERIES OF EXPERIMENTS

Fourteen cages with about 100 worker bees in each cage were prepared for feeding, the preparation of the cages and the placing of the food and water vials having been completed at 4 p. m. At the time when the cages were filled, many of the older bees were out of the hive and the younger bees taken tended to live longer than the average of the bees of the colony. So far as could be seen, there was no difference in the bees of the several cages. Beginning the next day and on each succeeding day the dead bees were removed from each cage at 4 p. m. and counted, as recorded in Table 1. Doubtless all these bees carried with them a certain amount of food when they were put into the cages. This food and their reserve apparently were sufficient to prevent death for an average of 1.74 days in the lot of bees to which no food was given, the last bee dying on the third day. It is therefore proper to conclude that when the bees lived more than three days or longer than an average of 1.74 days within the limits of the probable errors, they were able to utilize as food the material given them. Because of different conditions of temperature, the average length of life of the bees without food in the different series is unlike, and therefore it is undesirable to make detailed comparisons between different series.

The following plan was used in feeding the bees in the first series:

Lot No. 1.—No food given. Water at all times available (control cage).

Lot No. 2.—Glucose, 50 per cent solution.

Lot No. 3.—Levulose, 50 per cent solution.

Lot No. 4.—Sucrose (C. P.), 50 per cent solution.

Lot No. 5.—Honey from the apiary of the Bureau of Entomology, probably chiefly from tulip trees. This honey was dark in color and doubtless contained relatively large amounts of dextrin. No analysis was made. The percentage of dextrin was smaller than in the case of the honeydew honey used (lot 12).

Lot No. 6.—Lactose, 1 part in 10 parts of water.

Lot No. 7.—Raffinose, 1 part in 2 parts of water.

Lot No. 8.—Mannose, 50 per cent solution.

Lot No. 9.—Maltose, 50 per cent solution.

Lot No. 10.—Dextrin 1 part, sucrose (commercial cane sugar), 1 part, water 2 parts. Compare use of dextrin in the second series.

Lot No. 11.—Starch (potato) 1 part, sucrose (commercial cane sugar) 10 parts, water 10 parts. Compare use of starch in other series.

Lot No. 12.—Honeydew honey. This was collected in the apiary of the Bureau of Entomology then at College Park, Md., in 1909, removed from the combs by heating after crystallizing hard, and was then stored in tin cans. Because of the fact that this honeydew honey crystallized so hard, there is now reason to suspect that it contained melezitose. Since this trisaccharide is satisfactory as food for honeybees (see series 3), this possibility makes no special difference in the results of this test. Unfortunately by the time the presence of this sugar was suspected in honeydews gathered near Washington, all of the material collected in 1909 had been used.

Lot No. 13.—Brown sugar, commercial. No analysis was made. The purpose of using this material was to determine if possible whether the beekeeper is safe in using it as winter food for his bees. Under such circumstances the bees would be confined to the hive longer than in this series of feedings, and the high percentage of nonsugars would doubtless cause dysentery. A 50 per cent solution was used.

Lot No. 14.—Commercial glucose.

Table 1 shows the daily counts of dead bees in each cage, the average length of life, and the probable error of the length of life of these bees. Records were kept of the solid food consumed when food was given and in each case it was found that considerable quantities per bee per day were ingested. These records are not here given as the amounts are unimportant in the present discussion.

TABLE 1.—*Daily death rate and average length of life of bees on various foods, first series*

Food	Number of deaths after different periods (days)																	Total number of deaths	Average length of life (days)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
No food	32	62	6															100	1.7400±0.0377
Glucose	0	3	8	2	9	5	11	9	12	10	9	15	5	1				99	8.3636±0.2125
Levulose	1	2	5	5	10	5	11	6	4	6	9	17	9	5	2	1		98	8.9490±0.2488
Sucrose	0	7	1	14	9	10	8	10	8	13	10	5	4	0	2			101	7.6040±0.2156
Honey	20	0	0	4	2	12	2	3	6	8	4	6	11	10	9	3	1	101	8.8020±0.3383
Lactose	45	51	1	1	1	1												100	1.6500±0.0534
Raffinose	35	57	3	5														100	1.7800±0.0492
Mannose	4	14	30	41	8	3												100	3.4400±0.0709
Maltose	5	3	6	17	18	8	7	6	2	4	5	9	4	4	1	1		100	6.9200±0.2545
Dextrin, sucrose	6	7	13	16	14	12	11	3	10	6	2							100	5.3800±0.1756
Starch, sucrose	1	3	2	6	6	3	7	7	4	11	7	18	11	5	7	1	2	101	9.8713±0.2539
Honeydew	10	0	5	7	0	12	13	10	14	21	8							100	7.2000±0.2017
Brown sugar	2	15	12	13	9	12	22	10	2	3								100	5.1900±0.1540
Commercial glucose	5	7	17	25	29	12	3	3	1									102	4.3235±0.1059
Temperature, °C	23	23.6	24	23.5	23.4	23.4	20.6	20	19.5	21.2	22.1	21.2	21.6	22	22.8	23	20.6		

SECOND SERIES OF EXPERIMENTS

A second lot of cages of the same kind were filled with bees and again fed in the same way, the differences in food being noted later. In this series, the temperature conditions were somewhat better for longer life without food, as the bees were less active. There may have been slight differences in the amount of food carried by the bees when put into the cages, but this could not be determined. Attention should be called to the rather heavy death rate on the fourth and fifth days from unexplained causes, which tend to reduce the average length of life in this series. In spite of this the average and actual time of the death of the last bees were larger than in the first series. Counts of dead bees were again made daily.

Except as noted the foods given were as in the first series. The following feeding plan was used:

Lot No. 1—No food given (control).
Lot No. 2—Glucose.
Lot No. 3—Levulose.
Lot No. 4—Sucrose.
Lot No. 5—Honey.
Lot No. 6—Lactose.
Lot No. 7—Raffinose.
Lot No. 8—Mannose.
Lot No. 9—Maltose.
Lot No. 10—Dextrin, 50 per cent solution. (No sucrose added).
Lot No. 11—Starch. One part potato starch was heated in four parts of water at a temperature below boiling, the water content corrected, and the solution given the bees. The cooking may have changed a slight amount of the starch into dextrins and dextrose but most of it remained as starch. The purpose of the heating was to break up the starch grains and to make the food more easily ingestible.
Lot No. 12—Honeydew honey.
Lot No. 13—Brown sugar.
Lot No. 14—Commercial glucose.

Table 2 shows the results of the second series of feeding experiments. Except for changes in the foods given, these results may be compared with the data given in Table 1 covering the availability of the foods, but necessary allowance must be made for differences in temperature and other factors which caused slower death rates in the second series.

TABLE 2.—*Daily death rate and average length of life of bees on various foods, second series*

Food	Number of deaths after different periods (days)																						Total number of deaths	Average length of life (days)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		
No food	0	2	18	36	33	10	1																100	4.3400±0.0662
Glucose	1	1	9	28	15	6	4	14	6	8	6	2											100	6.1700±0.1797
Levulose	0	0	10	26	21	8	6	5	1	3	4	5	5	0	1	4	0	1					100	6.7400±0.2518
Sucrose	1	2	4	29	19	11	3	4	2	4	3	4	4	6	1	3	0	1					101	6.9604±0.2637
Honey	1	0	4	28	12	6	2	5	4	9	6	6	8	2	3	2	2						100	7.8200±0.2720
Lactose	5	14	15	48	11	6	1	1															101	3.7228±0.0866
Raffinose	0	3	13	28	36	18	2																100	4.5900±0.0727
Mannose	0	0	18	29	38	11	4																100	4.5400±0.0697
Maltose	0	1	6	33	19	4	4	4	7	1	0	5	6	3	2	2	1	0	1	0	0	1	100	7.0400±0.2850
Dextrin	0	7	28	29	27	8																	99	4.0101±0.0731
Starch	1	11	18	36	22	9	3																100	4.0600±0.0842
Honeydew	0	1	40	19	20	11	3	2	4	1													101	4.4257±0.1127
Brown sugar	0	0	18	43	19	10	5	3	2														100	4.5800±0.0921
Commercial glucose	0	2	20	44	11	7	4	6	1	3													98	4.6122±0.1183
Temperature, °C.	17.4	17.4	20	19	19.4	20	20	21.4	19.6	17.6	16.6	16.1	15.1	14.5	15	15.1	15	15				17.4		

THIRD SERIES OF EXPERIMENTS

The finding of the rare trisaccharide melezitose in honeydew honey collected in Pennsylvania in 1917 (*37*) led to a further search for this sugar in honeydew honeys. It was found that this sugar is occasionally gathered from coniferous trees near the apiary of the Bureau of Entomolgy in considerable quantities. A supply of purified melezitose was obtained for feeding experiments from the Bureau of Chemistry to determine whether honeybees are able to utilize this sugar as food.

Three lots of bees, each of 200 individuals, were placed in cages like those used in previous experiments September 29. One lot was given no food, one was fed on sucrose (commercial cane sugar, 50 per cent solution), and the third was given a 50 per cent solution of melezitose. Water was provided each lot of bees as in previous experiments. The bees were placed in the cages in the afternoon of September 29, and the dead bees were removed and counted daily as in previous experiments. In this instance the cages were kept in a room in the laboratory with diffused but without direct sunlight. It was necessary for the investigators to leave the laboratory October 28 for a considerable period, and it was therefore impossible to continue the counts after that date. The results covering the availability of melezitose as food were entirely conclusive, but the figures given can not be compared with those in other series. Fifteen bees still remained alive in the cage given cane sugar and one bee remained in the melezitose cage at the close of observations. Table 3 gives the data obtained.

It was noticed that the melezitose tended to crystalize about the feeder, and to remedy this the water bottle was shifted to the places where the crystals appeared, so that they might be dissolved and used by the bees. Throughout the experiment the bees fed on cane sugar were noticeably more active than those fed on melezitose, and this activity possibly hastened their death. The bees on cane sugar died at the average rate of 6.38 per day while those on melezitose died at the average rate of 6.86 per day. During the first 15 days 84 bees died on cane sugar and 140 on melezitose, or at the average rates of 5.60 and 9.33 daily, respectively. Between the twelfth and fifteenth days, during which no counts were made, an unexplained heavy death rate occurred in both cages.

TABLE 3.—*Daily death rate of bees on various foods, third series*

Food	Number of deaths after different periods (days)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No food	125	75													
Cane sugar	5	0	1	0	0	(1)	2	8	0	8	11	12	(1)	(1)	37
Melezitose	10	3	0	1	4	(1)	21	8	5	16	23	24	(1)	(1)	25

Food	Number of deaths after different periods (days)														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Cane sugar	8	7	12	6	(1)	16	2	8	(1)	6	9	(1)	14	13	
Melezitose	2	10	12	4	(1)	6	4	1	(1)	10	1	(1)	4	5	

[1] Dead bees not removed and counted.

FOURTH SERIES OF EXPERIMENTS

In order to extend these observations to other carbohydrates, a fourth series of feedings was undertaken. In general the methods were the same as for the previously reported series, except that the bees were not hand picked and a larger number of bees was used. There were some drones in the cages, but the number was small and these are omitted in the calculations. The bees were placed in the cages in the early afternoon, the operation being completed at 2.30 p. m., at which time the various foods were put into place. Cages 2 and 3 of this series contained bees without food, kept in other places to determine what effect increased activity has on the length of life of worker bees without food. This has been reported previously (*60*) and is here omitted.

The following foods were given the different lots in this series:

Lot No. 1.—No food given. Water at all times available.
Lot No. 4.—Glycerin, undiluted.
Lot No. 5.—Dextrin, 50 per cent solution.
Lot No. 6.—Inulin to which water was added without heat.
Lot No. 7.—Soluble starch in two volumes of water.
Lot No. 8.—Sucrose, 50 per cent solution.
Lot No. 9.—Galactose in 4 volumes of water.
Lot No. 10.—Lactose in 10 volumes of water.
Lot No. 11.—Raffinose in 2½ volumes of water.

The varying amounts of water in the food was necessitated by the comparative solubilities of the various substances. In such cases as soluble starch, for example, the material was not in actual solution, but the water present served to carry the food to the bees, and other water was available in all cages.

With the possible exception of glycerin the bees took carbohydrate when it was offered them. In the case of the glycerin, the humidity of the cellar in which the bees were kept may have caused moisture to be absorbed, and the volume of food had not noticeably decreased at the time the last bees died. Table 4 gives the results of the fourth series of experiments.

67589—27——2

TABLE 4.—*Daily death rate and average length of life of worker bees on various foods, fourth series*

	Number of deaths after different periods (days)																	Total number of deaths	Average length of life (days)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
No food	42	141	87	4														274	2.1934±0.0286
Glycerin	75	148	91	4														318	2.0755±0.0285
Dextrin	61	98	81	7														247	2.1377±0.0350
Inulin	79	181	51	1														312	1.9167±0.0248
Soluble starch	95	162	65															322	1.9068±0.0263
Sucrose	40	15	1	7	11	20	9	17	20	13	15	5	4	10	2	2	1	192	6.6719±0.2090
Galactose	63	93	66	4														226	2.0487±0.0359
Lactose	64	113	116	8	1	1												303	2.2475±0.0321
Raffinose	64	73	143	21														301	2.4020±0.0349
Temperature ° C	20.4	19.8	18.8	18.8	19.4	19.0	18.8	18.8	18.8	22.0	19.2	19.8	19.4	20.6	19.8	19.2	21.5		

FIFTH SERIES OF EXPERIMENTS

In this series a number of carbohydrates were introduced which probably never occur in the food of bees, but by means of which the presence of certain enzymes in the alimentary tract may be tested. These will be discussed later, but special reference may here be made to the use of trehalose. In this series one control cage of bees was given water while another cage received neither food nor water. Daily counts were made as in other series and in every case there was reason to believe that solid carbohydrate materials were actually ingested by the bees. It should be noted that if the bees had been able to hydrolyze some of these carbohydrates a death rate even more rapid than that of starved bees might have been expected, since when split these glucosides become poisonous. No such change in death rate was observed. Cages 1 and 2 of this series contained drones only and are not here discusssed, as they have been reported elsewhere (*61*).

The following food materials were tested in this series of experiments:

Lot No. 3.—Sucrose, in 2 volumes of water; no other water given.

Lot No. 4.—Amygdalin, in 2 volumes of water; no other water given.

Lot No. 5.—Salicin, in 2½ volumes of water; no other water given.

Lot No. 6.—Mannite in 2½ volumes of water; no other water given.

Lot No. 7.—Xylose, in 2 volumes of water; no other water given.

Lot No. 8.—Mannose in 2 volumes of water; no other water given.

Lot No. 9.—Rhamnose in 2 volumes of water; no other water given.

Lot No. 10.—Trehalose, in 2 volumes of water. At the beginning of this feeding 10 gm. of dry trehalose were available and by the third day half of this quantity had been taken by the bees and they were apparently living well on it. On the eighth day the last drop of food was removed from the feeder and 5 c. c. of water was placed in it to wash out any food that might remain. No other water was given. Whatever food the bees had from the eighth day until the last bees died must have been carried in their honey stomachs. The average length of life is therefore not fully indicative of the ability of the bees to utilize this sugar.

Lot No. 11.—*d*-Arabinose, in two volumes of water, no other water given.

Lot No. 12.—*l*-Arabinose, in two volumes of water, no other water being given.

Lot No. 13.—No food (control), water given in a feeder.

Lot No. 14.—No food, no water given.

Table 5 shows the daily death rate and the average length of life of the bees in this series of experiments, and the probable error of the average. Because of the unusual character of some of the materials offered to the bees in this series, it seemed desirable to force the several carbohydrates on them by providing no water other than that in which the carbohydrates were dissolved. In every case material was removed from the feeder bottles, indicating that carbohydrates were actually ingested. It may safely be assumed that at least some of these materials were not at all attractive to the bees, but since some of the material was ingested in each case, it may further be assumed that if it had been hydrolyzed the length of life would have been modified either by hastening death or by prolonging life. The rather

surprising result from the use of trehalose will be discussed later. The absence of water from cage 14 seemed to make no appreciable difference in the length of life of bees without food, since the average length of life in cages from which water was withheld was actually slightly longer than for bees without food to which water was supplied. This difference is of course too small to have any significance, since the average length of life of bees in several cages that starved in spite of the fact that they took carbohydrates, slightly exceeded the average length of life for bees without food and without water.

TABLE 5.—*Daily death rate and average length of life of worker bees on various foods, fifth series*

	Number of deaths after different periods (days)																		Total number of deaths	Average length of life (days)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Sucrose	5	3	17	8	20	9	34	22	47	41	38	24	23	16	6	1	4	0	2	320	9. 1750±0. 1287
Amygdalin	10	78	171	33	1															293	2. 7850±0. 0274
Salicin	31	75	171	17																294	2. 5918±0. 0296
Mannite	15	62	141	108	18	4														348	3. 1839±0. 0350
Xylose	13	81	204	24																322	2. 7422±0. 0244
Mannose	37	100	110	119	15															381	2. 9344±0. 0365
Rhamnose	70	134	176	18																398	2. 3568±0. 0277
Trehalose [1]	24	8	13	25	31	32	30	10	24	37	30	14	31	15	9	2	1			336	8. 0268±0. 1456
d-Arabinose	34	52	168	41	1															296	2. 7399±0. 0330
l-Arabinose	22	89	181	34	1															327	2. 7034±0. 0281
No food	24	175	175	3																377	2. 4164±0. 0216
No food, no water	14	90	226	42																372	2. 7957±0. 0238

[1] See explanation in text of the food supply on this sugar.

SIXTH SERIES OF EXPERIMENTS

Two lots of bees were placed in the small cages November 24, one being provided with a paste made of white clover honey and soluble starch (121 bees) and the other with a paste made of soluble starch and cane sugar (169 bees). Both lots of bees were given water in a small feeder. The bees were kept in a laboratory room with subdued light at ordinary room temperature. The purpose of these feedings was to follow the starch through the alimentary canal and not to establish the death rate. The bees on honey and starch died somewhat faster than those on starch and sugar, probably because they were able to take more food and thus to clog their alimentary tracts more rapidly. The last bee living in the cage with sugar and starch was removed alive December 4, while the last bee on honey and starch was removed November 29. The paste of sugar and starch tended to glaze, so that the bees could not readily take the food. To overcome this difficulty a drop of water was placed on the paste from time to time. The bees immediately took the food more readily. The details of the examinations of this series will be treated under the discussion of starch. The "soluble starch" used was tested before using and every grain examined stained deep blue with iodine.

THE AVAILABILITY OF THE CARBOHYDRATES AS FOOD FOR HONEYBEES

From the foregoing records, it will be seen that in certain cases the bees died as quickly after ingesting certain carbohydrates as they did when given no food. This fact is interpreted to indicate that these carbohydrates are unavailable as food, even though actually ingested. In other cases length of life was materially increased by the eating of carbohydrates, which is interpreted to mean that such carbohydrates serve as food for the bees and are partially, and probably in most cases almost entirely, utilized. The results of experiments in which mixed foods were given indicate that not all components of the material can be utilized.

The materials used in these experiments vary greatly in taste to the human tongue. That a carbohydrate tastes sweet to man is not proof that this is true for bees, and some differences in taste to the bees may have made an appreciable difference in the amount of material ingested and may therefore have influenced the length of life. In some cases the bees took very small amounts of the material available to them and may actually have starved. In all cases they took some of the material offered (except possibly glycerin), and unless the carbohydrate served as food they died as if no food were given.

In certain cases, such as the glucosides, the material was offered the bees as a means of determining whether they are able to hydrolyze it. In such cases it is assumed that if hydrolyzation were accomplished the length of life would actually be decreased rather than increased, since the products of the splitting of these materials produces a material known to be poisonous to insects.

A summary of the results of these tests follows:

RESULTS OF EXPERIMENTS

BEES WITHOUT FOOD

The controls for these tests were bees which had no food other than that which they carried in their honey stomachs at the time they were placed in the cages. In the second series the bees lived without food for 4.34 days,[8] while in the first series the average length of life was only 1.74 days. The difference in temperature doubtless accounts for such variation. Later experiments gave still different results, but in no other case did the starved bees live as long as in the second series of feeding experiments. For this reason, the results of all feedings in the second series are less definite than those in other experiments.

In colonies wintered in cellars with proper temperature conditions bees have been known to live for several weeks when separated from their food supply. Under the conditions surrounding the bees in these tests however, an average length of life of 4.34 days seems high. In other experiments (*60*) it has been found that high temperatures and increased activity greatly increase the death rate. In certain cases it was found that some bees actually died of starvation within a few hours after being put into cages, the food in the alimentary tract being wholly exhausted. In other experiments not here recorded, the quantity of food consumed by bees hour by hour was determined by weighing, and an astonishing quantity was found to be consumed when the bees were at all excited, and their metabolic activity thereby increased.

In considering the availability of each carbohydrate used it is necessary to compare the average length of life (and to some extent the greatest length of life observed) with the average length of life of the bees without food in the same series of feedings, since in each feeding series the various lots were subjected to the same external conditions, so far as known.

GLUCOSE

The bees lived well on this sugar as would be expected from the fact that it is one of the chief constituents of honey. The results of the second feeding were less conclusive than those of the first feeding, but the differences in these results are insufficient to suggest any difficulty with glucose as a food. Glucose being a simple aldohexose sugar, no enzyme is required to make it available for absorption. It is evidently assimilated readily.

LEVULOSE

As would be expected, this sugar served as food for the bees in both feedings (series 1 and 2). In the first feeding the bees lived as long as those fed on honey, within the limits of the probable error, and only a slightly shorter time than those on the cane sugar and starch mixture. In the second feeding they lived slightly less time than those on honey and as long as those on maltose, within the limits of the probable error. This being a simple ketohexose sugar, no enzyme is required to render it available for absorption. It is evidently readily assimilated.

[8] In the discussions probable error figures are omitted but they will be found in the tables for each series of feedings.

GELACTOSE

This sugar was fed only in the fourth series. The bees were not nourished by it and died as rapidly as the bees without food, within the limits of the probable error. This is a simple sugar and no enzyme is needed to render it available for absorption, so that apparently it is unsuitable for assimilation by honeybees. This simple aldohexose sugar occurs as a constituent of lactose and of raffinose and in more complex compounds, and attention may be directed to the unavailability of lactose as food for bees (p. 25).

MANNOSE

This simple aldohexose sugar does not occur in nature in simple form and therefore would not be expected normally to form part of the food of bees. It was tested in series 1, 2, and 5. In series 1 the bees on this food lived 1.977 times as long as did the bees without food, or 15.65 times the sum of the probable errors. In the second series they lived 1.046 times as long as the bees without food, or 1.47 times the sum of the probable errors, while in the fifth series the mannose bees lived 1.214 times as long as the starved bees, or 8.915 times the sum of the probable errors. In the first series the last bee on mannose lived twice as long as the last bee without food; in the second series the last bees of the two lots died the same day; and in the fifth series the last bee on mannose lived one day longer than the last bee without food.

The mannose used was α-dextro-mannose. No enzyme would be required to split this sugar so as to make it available for absorption, and although on the whole the bees on this food were able to live somewhat longer than starved bees (in all cases in which the length of life was greater than the sum of the probable errors and in two cases in which it was several times the sum of the probable errors) it still appears not to be readily available for absorption and assimilation. So far as the author has been able to learn, this sugar has not been tested with other species of animals. Mannite, an alcohol, will be discussed later.

SUCROSE

The first disaccharide to be considered is sucrose, which occurs widely in nature, as in cane and beet sugars and in nectar. This sugar was used in all of the first five series of feedings, serving as a control for the other carbohydrates used. The bees lived well on it in all cases.

Cane sugar is used extensively by beekeepers to supply their bees when there is a shortage of honey, and is used especially for winter food when the honey in the hive is of inferior quality (indicating a high percentage of dextrins). It is well known, therefore, that bees are able to use this sugar as food. Since it is often maintained, however, that bees need the salts and other materials in honey which are lacking in highly refined cane sugar, the only question which ever arises in the minds of beekeepers is whether cane sugar is as good as honey for use in the spring when brood is being reared abundantly. There is nothing in the results of these experiments to throw any light on this subject.

By the action of the enzyme invertase (sucrase) each molecule of sucrose is hydrolyzed to form one molecule each of glucose and levulose, both of which, as has been seen, are available for assimilation

by bees. In feeding sugar sirup, beekeepers often partially invert the sugar by the use of an organic acid (usually tartaric) and heat. The purpose of this partial inversion, however, is not to make the sugar assimilable but to prevent the formation of crystals which the bees can not use without waste. As the result of partial inversion of the sugar before feeding, followed by still further inversion from the manipulation of the sirup by the bees during storage, a balanced supersaturated sirup is formed which will remain liquid for months.

Invertase is exceedingly abundant in nature, being found in many species of plants and animals. That this enzyme is present in bees has been known for many years (*4, 58, 59, 67, 82, 83*). Several years ago Doctor Hudson, formerly in charge of the carbohydrate laboratory of the Bureau of Chemistry, was able to extract invertase from all parts of bees that had been dead for some time, and the amount of the enzyme present was found to be large (unpublished data). Invertase is also present in many pollens (*31, 39, 57*), but probably not in all. Invertase is also present in honeys (*23, 24, 25, 46, 59*). This is indicated by an interesting beekeeping practice recommended years ago by Doolittle, of putting unheated honey in sugar sirup to be used for winter food "to prevent granulation."

According to Hudson and Paine (*36*), invertase fails to act in alkaline solution and rises to a maximum in weakly acid ones. The PH value of the normal ventriculus of the worker bee is 6.8 to 7 (neutral). It is about the same for the rectal ampulla, except when the latter is heavily loaded with pollen, in which case the PH value may be as low as 6.4.[9]

LACTOSE

This disaccharide sugar is a constituent of milk and by the action of the enzyme lactase is hydrolyzed into the monosaccharide sugars, galactose and glucose. It was tested in series, 1, 2, and 4. In each case the bees died as if starved, and in two cases sooner. It will be seen from the discussion of galactose that bees are not nourished by that sugar. If lactose were hydrolyzed by bees, there would be reason to expect that the glucose constituent would serve as food for them, regardless of whether or not the galactose is utilized. Lactase is also from its composition a β-galactoside (*70*) and compounds of this group are hydrolyzed by emulsin, which is sometimes considered as consisting of a mixture of at least four distinct specific enzymes (*6*). The failure of the bees to utilize lactose therefore suggests the absence of emulsin, as is also indicated in other tests with glucosides.

In all tests with lactose the bees died as soon as if they had been given no food, so that they are evidently not nourished by it. In the first series of feedings they took almost no lactose, but they did take somewhat more in the second. Some lactose was taken in the fourth series, but the quantity was not weighed. Because of the low solubility of lactose, it was necessary to make a dilute solution. The results indicate, however, that this was not the cause of the death of the bees.

MALTOSE

In both feedings (series 1 and 2) the bees lived considerably longer, 3.977 and 1.622 times, respectively, on maltose than did the bees without food. In the first feeding they lived as long as did the bees

[9] Determination made by A. P. Sturtevant.

on honeydew honey, within the limits of the probable errors, and in the second feeding they lived slightly longer. They are evidently able to utilize this carbohydrate as food.

Maltose is a stage in the transformation of the insoluble and highly complex polysaccharide starch into the simple monosaccharide glucose. This action in nature is brought about by the action of enzymes of the amylase group, but it is usually believed that there are several distinct enzymes, each one of which is limited in its action to a particular stage of the series of changes which occur. The original action is variously described as either the formation of dextrin and maltose, or of dextrins alone. If dextrins are formed then there is a second stage in the action in which maltose is formed. After maltose (a disaccharide sugar) is once formed, by whatever steps, it is further hydrolized by the action of maltase (glucose) an enzyme of the invertase group, each molecule of maltose forming two molecules of glucose. The enzymes of the amylase group are not concerned in this action. The enzyme necessary for the splitting of maltose (maltase) is evidently present in bees.

The results obtained in feeding maltose are especially interesting in view of the inability of bees to utilize dextrins and starch, as will be later shown. Maltose is the first step in the transformation of starch into dextrose which the honeybee seems to be able to utilize as food. There was doubtless a small quantity of dextrin in the material fed but this, as will be seen later, is unavailable to bees.

TREHALOSE

This is a disaccharide sugar which is hydrolyzed into two molecules of glucose. It does not reduce Fehling's solution and is one of the most difficult sugars to hydrolyze with acids. It is not acted upon by the enzymes maltase, invertase, emulsin, or amylase but requires a specific enzyme, trehalase. Because of the fact that the bees were able to utilize this sugar in series 5, it seems necessary to discuss the distribution and splitting of this rare sugar at some length.

This sugar was first noted by Wiggers (*77*) [10] in 1832, when he observed crystals from the water extract of ergot on rye, which he called Mutterkornzucker. It was differentiated from other sugars by Mitscherlich (*54*) in 1856, who gave it the name mycose. He determined the formula and described the crystal form. In 1858 Guibourt (*32*, *33*) described the trehala manna produced by the weevil *Larinus nidificans* on the twigs of a plant of the genus Echinops in Persia.[11] The material which Guibourt studied was turned over to Berthelot (1857–1859) (*7*, *8*, *9*), who extracted from it a sugar which he named trehalose, the name still commonly used, although it has since been determined that it is idential with the sugar seen by Wiggers (*77*) and Mitscherlich (*54*). Beginning in 1889, Bourquelot published a large series of papers on the finding of trehalose in various fungi, among which it is widely distributed during the period of rapid growth. The sugar disappears rapidly after the picking of the fungi and is replaced by mannite. It also occurs in myxomycetes, in ferns, in certain marine algae, and in the

[10] This reference and some others which from their nature were not consulted by the author, are taken from an unpublished manuscript which the author was privileged to consult. HARDING, T. S. BIBLIOGRAPHY CONCERNING TREHALOSE. [Unpublished manuscript in U. S. Dept. Agr., Bur. Chem. Carbohydrate Lab.]

[11] Regarding this insect and its product, see (*64*).

resurrection plant, *Selaginella lepidophylla*, of Mexico and western Texas. A sugar, iso-trehalose, has been artificially produced by Fischer and Delbrück (*29*).

This list of the known sources of trehalose has been given to show the improbability that this sugar has ever served as a natural food for honeybees. Apparently trehalose replaces sucrose in those plants which contain no chlorophyll and which do not manufacture starch. It serves as a reserve food material which can be utilized by these plants only after hydrolysis of the sugar into glucose. It is, of course, found in some green plants, as has been mentioned, but it has so far not been found in any plant from which honeybees make collections of sugar in the form of nectar. It is probable that other sources of trehalose may be discovered.

The inversion of trehalose into two molecules of dextrose was first described by Bourquelot. He found that trehalose was not affected by the invertase of brewer's yeast or by emulsin. With maltase he encountered some difficulties, for the enzyme obtained from Aspergillus split both maltose and trehalose. By the application of heat he was able to show that the ferment ceased to act on trehalose at 64° C., while it continued to act on maltose up to 74°, thus proving that two separate enzymes are present. It appears from Bourquelot's work, therefore, that for the splitting of trehalose by natural means a specific enzyme, trehalase, is required. It is important to note that trehalose, unlike sucrose, is split only with difficulty by the use of dilute mineral acids (*50*, *79*, *80*), and for the complete splitting into glucose only two hours' heating at 110° in the presence of 5 per cent sulphuric acid is necessary. It is not to be believed that the splitting which evidently occurred in the utilization of this sugar by the honeybee is due to the presence of acids in the alimentary tract, as appears to be the case in the splitting of inulin in man. Fischer (*28*) questions Bourquelot's conclusion of the necessity of a specific enzyme, since when one part of trehalose was kept at 33° for 40 hours with 5 parts of dry pure yeast and 10 parts of water containing 2 per cent thymol, 20 per cent of the reduced sugar was formed. He also found that trehalose is hydrolyzed by amylase, but because of the uncertainty which still surrounds the various enzymes, Fischer makes no positive statements regarding these facts.

In considering the results of Fischer, Bourquelot and Gley (*15*, *16*) published two small papers giving data that appear to be conclusive proof that a specific enzyme is required for the splitting of trehalose. They found that although dog-blood serum and human urine contain a very active diastase, as they showed for the particular materials used in their experiments, those substances are without action on trehalose, and therefore the diastase of malt is a mixture of enzymes containing both diastase and trehalose. They also found later that beef-blood serum was without action on trehalose. In their later experiments they found that trehalose is hydrolyzed in the intestine of the adult rabbit, but that there was no reduction from the action of the pancreas. Extracts of pancreas and intestine of a young dog gave negative results.

Before the bees were fed trehalose, its utilization unfortunately was not predicted and an inadequate amount of the sugar was obtained. Only 10 gm. were available. As a result, the average length of life is not found to be quite so great as in the case of the

bees fed sucrose during the same series of feedings. Before death occurred for all the bees, however, the supply of trehalose was entirely exhausted, and the last bees doubtless died of starvation. In the notes made at various times during the course of the experiment, it is recorded that the bees were as active as those on sucrose. Eight days after the first feeding, when the supply of trehalose sirup in the feeders was exhausted, the bees were in fine condition, and the last bee did not die until 17 days after the first feeding. The last bee on sucrose did not die until two days later, but this lot of bees took more food (because it was available), and this is doubtless the explanation of the longer life of the sucrose-fed bees. It appears from the records of this experiment that the bees on trehalose lived as well on this sugar as did the bees on sucrose. The contrast in series 5 with the bees on various foods that are not utilized and with those to which no feed was given is most striking.

It appears, therefore, that whatever enzyme is necessary for the splitting of trehalose, that enzyme is found in the alimentary canal of the honeybee. If, as is usually assumed a specific enzyme is required for this process, then in some fashion the honeybee has elaborated this. In view of the peculiar distribution of this sugar in nature as at present known, this result is somewhat surprising.

The only insect other than the honeybee which might possibly use trehalose as a food is *Larinus nidificans* which produces the trehala manna and the larvae of various species of Diptera which live on various fungi. In view of the fact that trehalose, which is later used by it in the manufacture of manna, is found in such large proportions in the rectal content of the Larinus larva, one might venture the guess that this larva does not digest trehalose. There seems to be no available information concerning the ability of the various larvae found in fungi to digest the several carbohydrates found therein.

From the feeding results, it is suggested that bees contain the enzyme trehalase. The actual finding of an enzyme in an organism which has probably never encountered the substance acted upon until it fell into the hands of an experimenter, is not a new thing. Lactase has been found in the emulsin of almonds, or at least an enzyme has been found which hydrolyzes lactose, although it may act on the glucose element (*1*). Emulsin has been found in alimentary canals of vertebrates where it may produce toxic substances and where it is apparently not a useful enzyme. Bierry (*12*) (cited by Armstrong (*1, p. 125*)) found an enzyme which can hydrolyze α-methyl galactoside in the digestive juices of Helix, although no compound of α-galactose is known in nature. Kondo (*43*) "found that the liver is capable of synthesizing amino-acids from the corresponding ketonic acids and ammonia, even when the acids in question are not such as occur in the proteins of the organism. Since the one isomere is not of natural occurrence, but notwithstanding this it is attacked, it follows that, if a special enzyme is required, these organisms have produced in the course of their evolution an agent which has never had a chance of exerting its activity until the organisms fell into the hands of the physiologist" (*6, p. 137*). This quotation from Bayliss is here given because it seems probable that the hydrolysis of trehalose by the honeybee is another instance of the evolution of the ability to produce an enzyme, assumed to be

specific. It is probable, however, that never before in the evolution of the honeybee has trehalose been used as food, under which condition the enyzme could be utilized. Unless this is the case, one might assume that there is a source of trehalose as yet unknown which bees have at some time utilized as food; but from the character of the places where trehalose has so far been found, this seems rather improbable. However, since bees have been found collecting and utilizing melezitose (p. 16), one need not be surprised to find that they have used almost any carbohydrate at some time or another.

RAFFINOSE

In three feeding series (1, 2, and 4) the bees died about as soon on this sugar as did those without food. The difference is practically within the limits of one time the probable errors of the experiment. Bees are evidently not nourished by this sugar, although in both feedings they took considerable quantities, more than in the case of lactose, and even more per bee per day than in the case of some foods which maintained life for a time.

Raffinose belongs neither to the saccharose nor to the glucose groups of sugars but is a sacchoid ($C_{18}H_{32}O_{16}$) or trisaccharide. By hydrolysis it is first split into levulose and melibiose, and by further hydrolysis the melibiose is split into glucose and galactose. The first splitting is presumably due to the action of invertase, the same enzyme which splits sucrose into levulose and dextrose, and which is so abundant in bees. If this action occurs in the case of raffinose fed to bees, it might be expected that the bees would be nourished by the released levulose. The second enzymic action is brought about by the specific enzyme melibiase, and as melibiose alone was not fed to bees there is nothing in these feedings to show whether the second enzyme is present. Melibiose divides into the same sugars as are produced by the action of lactase on lactose. One can at present draw no definite conclusions concerning the failure of raffinose to keep the bees alive.

MELEZITOSE

In the one feeding of melezitose (series 3) the bees lived well and were evidently able to utilize this sugar as food. While the death rate was slightly more rapid than in the case of bees fed commercial cane sugar (sucrose), the fact that one bee was still alive at the end of a month is definite proof that bees utilize the sugar.

On hydrolysis, melezitose is converted into turanose and glucose, and by further hydrolysis turanose yields glucose and levulose. Hudson and Sherwood (*37*) found that invertase is without any effect on a pure solution of melezitose. The enzyme which is capable of hydrolyzing this trisaccharide is not determined. By the use of acids the first step in hydrolysis is comparatively easy, but considerable difficulty is experienced in hydrolyzing turanose by means of acids. This is of interest in the present instance as it yet remains to be determined whether the bees simply hydrolyzed the melezitose to obtain glucose which nourished them, or whether they completed the process and hydrolyzed the turanose as well. The sluggish condition of the bees during the feeding suggests but does not do more than suggest, that they were accumulating turanose as feces. Turanose is hydrolized by an enzyme found in *Aspergillus niger* known as turanase.

DEXTRIN

In the first feeding in which dextrin was given with cane sugar the average life of the bees was longer than in the case of bees without food. This mixture maintained life longer than commercial glucose, which contains relatively more dextrin than the food used, and was about the same as for brown sugar, which contains undetermined indigestible materials. In the second series, however, the cane sugar was omitted and the bees died sooner than did those without food, but within the probable error. The results in series 4 are the same as in series 2. Bees are evidently unable to maintain life on this carbohydrate. The dextrin used gave a slight blue color with a reddish tint when tested with iodine in potassium iodide. As there are doubtless many forms of dextrin in the transition of starch into maltose, it is impossible to say at just what stage of the hydrolyzing process in starch there is obtained a carbohydrate available to bees; but it is evident from the results of this feeding, as well as from other evidences, that most dextrins are not available to bees.

The inability of bees to utilize dextrin as food is a matter of primary importance in practical beekeeping. In the work done by Demuth and the author on wintering of bees, it was found that it was possible to cause the condition known as dysentery in wintering bees merely by giving honeydew or any dark honey and by confining the bees to the hive for long periods. The chief differences between the contents of dark honeys and the contents of lighter ones, on which dysentery is not contracted, are to be found in the dextrin content, although other materials are present in somewhat larger quantities in dark honeys than in lighter ones. In all this discussion an exception must be made of buckwheat honey which is quite satisfactory as a winter food for bees. The dark color in this honey presumably comes from tannin bodies, doubtless present in small quantities and not known to be injurious to bees. These facts suggested that dextrins in honeys are the actual cause of dysentery, and further suggested that in no case is any microorganism involved, as has been suggested. To determine the availability of dextrin, the first series of experiments here recorded was undertaken. Since in the first series dextrin was fed with sucrose, the result was inconclusive, and a second series was undertaken, in which, as has been seen, the bees died on dextrin as soon as if starved.

The literature on the digestion of bees, to which references have been made earlier, indicates that bees are able to digest starch, since the enzyme diastase has repeatedly been recorded as occurring in the alimentary canal of the bee. The term "diastase" is used in these cases presumably to include the whole series of enzymes which are capable of hydrolyzing starch into glucose, and dextrins are part of the series of compounds through which the starch passes in this series of changes. If therefore diastase is present in the bee one might expect that dextrin would serve bees as food. These matters will be treated at greater length under the discussion of the utilization of starch, in which it will be shown where the errors in former investigations have apparently been made.

Dextrins are the first soluble materials formed in the hydrolysis of starch in the plant, in which stage the carbohydrate material of the plant may circulate through plant tissues. This apparently

enables dextrin to pass through nectar glands and thus to enter the hive. If dextrin were actually hydrolyzed by bees, there might be reason to expect that in the honey-ripening process, this material would be changed into glucose. The variation in the dextrin content of honeys has already been mentioned. The presence of dextrins in ripened honeys and honeydew honeys is in itself circumstantial evidence of the inability of bees to provide the enzyme necessary for this transformation, at least in the honey stomach and salivary glands. As has been shown in the discussion of maltose, the enzyme which acts in the change of dextrin into maltose belongs to the amylase group, and apparently this entire group of enzymes is absent from the lumen of the alimentary tract of the bee. This will be discussed in detail under starch, in which discussion it is shown that amylase (or an allied enzyme) may be present elsewhere than in the lumen of the alimentary canal, and may thus help to explain the discrepancy between these results and those obtained by investigators who made enzyme extracts.

From these results the theory that dextrins are the cause of dysentery in wintering bees is greatly strengthened. Dysentery is so important in practical beekeeping that further investigations on this subject may well be carried on, especially since the work here recorded throws doubt on the presence of amylase or dextrases. It is certain, regardless of the final outcome of the work, that there is something in the darker grades of honey which is indigestible and that the accumulation of this material causes the condition so much dreaded by beekeepers. The theory of causation by a microorganism has so far nothing to support it. Of all the substances which increase in amount with the darkening of honeys, none is so evident as the increase in dextrins, which adds greatly to the validity of the theory that dextrins are the cause of dysentery. The various plant-coloring materials which occur to a greater extent in dark honeys, in association with dextrins, seem to be quite insufficient in quantity to account for the relatively enormous accumulations of feces in bees with dysentery; and that the quantity of feces to be accounted for is very large is well known to all beekeepers.

STARCH

This a complex polysaccharide, formed by the action of enzymes from many molecules of glucose, the starch molecule being exceedingly complex, and the number of constituent glucose molecules uncertain or variable. Starch is insoluble in water and in order that it be absorbed after digestion it must first be hydrolyzed by enzyme action (or by heat or acid artificially) until it reaches the stage of glucose again. That it may pass from place to place within a plant, it must be reduced at least to the stage of soluble dextrins. This reduction occurs in nature also through the actions of enzymes. As was stated under the discussion of dextrins, this cycle of enzyme action is presumed to depend on the successive action of several enzymes, all grouped together in the series of amylases (commonly called diastases). Starch grains are protected in plant tissues by a coating of cellulose, still more complex in molecular arrangement than starch itself. In order that the starch grain may be acted upon by amylase this coating must first be removed, either by the action of an enzyme, a cytase, or by other means. In the second series of

feedings, the coating was removed by cooking, while in the sixth series soluble starch which had been changed by some process that broke up the original starch grains into smaller units was used.[12]

In the first series, when potato starch was given with cane sugar, the average length of life was slightly greater (5.67 times that of the starved bees) than on any other food and the last bees died on the seventeenth day, at which time the last bees on honey also died. In the second series, potato starch was given in the form of a cooked paste and on this material the bees died as rapidly as if starved. The bees having dextrin died almost at the same rate as those on starch. This is interesting since, as observed in the discussion of dextrin, it is usually stated that bees have diastase in the alimentary canal. Petersen (*59*) states that he found diastase, but in his experiments he records nothing about the removal of the contents of the alimentary canal before the extraction of the enzymes; and because pollen is such a prolific source of diastase, the author was at first inclined to believe that the error in Petersen's work arose from the enzymes extracted from included pollen.

Since Petersen has stated positively that the bees contain within their alimentary canals the enzyme which serves to hydrolyze starch, it is desirable to examine his work in some detail. When he fed a solution of starch and sugar (heated for a time) which the bees accepted readily (see first series of experiments, p. 11), by means of iodine in potassium iodide, he traced the starch through the alimentary canal, found little of it in the rectal ampulla, and assumes that "a larger part" of it was converted into maltose or glucose. He was unable to demonstrate any change of starch into more soluble carbohydrates in the honey stomach, which is interesting in view of the claim of von Planta and others that there is diastase in honey. Not satisfied with the work previously done on this point Petersen performed the following experiment:

One gram of starch and 2 grams of NaF were dissolved in 100 c. c. of water with heat (solution I). Then 50 bee intestines were ground with 10 c. c. of equal parts glycerin and 1 per cent NaF solution, treated with toluol, and filtered. The filtrate 7 c. c. in quantity was brownish yellow and cloudy. He then made the following combinations:

(1) 3 c. c. filtrate and 1 c. c. solution I.
(2) 2 c. c. filtrate and 0.5 c. c. solution I.
(3) 2 c. c. filtrate and 0.5 c. c. solution I.
(4) Starch, solution I.

After 24 hours at room temperature No. 1 combination gave a red violet color with potassium iodide. After 24 hours more all four were tested with the following color results:

(1) dark rose; (2) light rose; (3) dark rose; (4) blue.

No. 1 was then allowed to stand at a temperature of 40° C. and after 24 hours more gave a light red color with potassium iodide. As the result of these experiments Petersen states: "I consider the

[12] According to Jost (*38, p. 149–150*), the making of a paste of starch changes its chemical composition, as well as the physical appearnce. "Employing the iodine test," he says, "we find that the original blue reaction rapidly gives place to a wine-red coloration. Finally, this latter reaction also disappears. Even without using iodine the fluid exhibits a marked alteration in appearance. Originally it is semi-fluid and opalescent; now it becomes transparent and quite watery. The starch, as such, has disappeared, and dextrin and maltose take its place. Maltose betrays its presence by the fluid being capable of reducing alkaline copper sulphate (Fehling's solution)."

breaking up of starch into dextrin as proven." He did not succeed in dissolving starch with the intestinal contents in a hanging drop.

The essential error in this experiment is that the author seemingly did not remove whatever pollen may have been in the intestines of the bees which he used in these experiments, and as most pollens contain diastatic enzymes, the whole experiment seems valueless.

After these experiments had progressed to series 4, the paper by Pavlovsky and Zarin (*58*) appeared, in which the statement was again made that the alimentary tract contains diastase. These investigators avoided the error which caused the present author to doubt the results obtained by Petersen, by removing the contents of the tract before extracting the enzyme. This necessitated still further work on starch, as a result of which the sixth series of feedings discussed in this paper was undertaken.

The feedings described for the sixth series were conducted, not to determine the death rate but to follow the course of ingested starch to ascertain whether any of it is modified by the digestive processes of the bee. To assist in the action of amylase, if present, these bees were not kept in a cool room but in ordinary room temperature[13]. They were not kept in a dark room but were exposed to a certain degree of stimulus from diffused, subdued light in order to induce somewhat greater activity and thus an increased need for food on the part of the bees. From time to time individual bees were removed while still living and the entire length of the alimentary canal was examined with Gram's iodine solution to determine whether any of the grains of soluble starch were in any way changed, as indicated by a change in color reaction with iodine.

If Pavlovsky and Zarin (*58*) are correct in stating that amylase is present in the lumen of the alimentary tract, then some of the grains of starch ingested as was the case in this series should stain red with iodine, others should take no stain with iodine, and still other grains should dissolve because the carbohydrate material has reached the stage of solubility. Whatever starch was actually rendered soluble would be invisible by the methods here used, but if diastatic action were actually occurring one should be able to find some starch grains taking a red stain with iodine and others taking little or no stain. If, on the other hand, every grain of starch stains deep blue with iodine, the conclusion seems obvious that the starch is not being acted upon by an enzyme.

Without going into the details concerning the examinations of individual bees, which were made several times a day as long as the bees lived, it may be said that up to the last day with the last bee, every ingested starch grain stained deep blue with iodine. It sometimes happened that the iodine would not at once penetrate the great mass of starch in the ventriculus and that the inner grains would therefore remain unstained or very slightly stained. Only the application of more iodine solution was necessary, however, to stain all the grains in the manner characteristic of unmodified starch.

[13] The temperature conditions prevailing in all these experiments were not especially favorable for the action of amylase. According to Jost (*58*) (figures after Kjeldahl (*40, p. 121*), at least for the amylase ferments found in plants, the action at 20° C. is about half that at 50° to 63° C. The maximum, minimum, and optimum points for different enzymes of the amylase group are not always the same. The temperature of the warmest part of a bee hive (consequently the approximate internal temperature of the bees in the hive) rarely exceeds 35° C. Bees away from the hive have a body temperature approximately the same as that of the surrounding air, varying between about 12° C (at which temperature flight may begin) and 35° to 38° C.

This was true of the few grains of starch found in the honey stomachs of a few bees and of all grains in the ventriculus, small intestines, and rectal ampulla; and even the hindermost grains, probably those longest exposed to whatever enzyme action was present, stained the same as those just ingested. The conclusion is therefore unavoidable that this starch is not modified by an enzyme present in the lumen of the alimentary canal of the worker bee.

When reputable investigators announce the finding of diastase in the alimentary tract, and there can be no question of the accuracy of their statements; when one can trace starch through the entire alimentary tract and find none of it modified; and when bees having starch alone die as rapidly as if starved, there must of course be some explanation of the difference, and this explanation becomes unusually interesting. For several days during the progress of feeding in series 6, no suggested explanation was found. When the contents of the alimentary canal of one of the bees in series 6 was dissected the entire ventriculus was left on the slide and the iodine solution allowed to run about it. Some of the ventriculus lying in the microscopic field was found to be peculiarly stained. The muscles surrounding the ventriculus stained deep reddish brown, indicating the presence on these muscles of stored glycogen. While apparently there is still much work to be done on the enzyme reactions of glycogen, it is a common opinion that the enzyme splitting glycogen into glucose is the same enzyme which splits starch into glucose. Probably several enzymes are necessary in either case, but presumably also the entire series of enzymes are the same in both cases. It is further believed that an enzyme which can hydrolyze a carbohydrate also has the ability to cause that same carbohydrate to be built up from the component sugar molecules. In other words, enzymes are known to possess reversing action. On this assumption, the presence of stored glycogen in the muscles surrounding the ventriculus indicates the presence in these muscles of a diastase which enables the tissue to form glycogen. Obviously there could be no stored glycogen in these muscles without the action of the necessary enzyme.

Pavlovsky and Zarin (*58*) took the precaution to remove the contents of the alimentary canal, thus getting rid of the pollen therein contained, which is a prolific source of amylase. Herein they were on safer ground than Petersen (*59*) who says nothing about the removal of the contents. The Russian authors did not, however, remove the surrounding muscle layers from the alimentary canal, which would in fact be an impossibility, since the muscles are definitely incorporated in the tissues of the walls of the ventriculus. The presence of glycogenase in muscles is, of course, not at all surprising, certainly not in the bee, since it is well known that tissues of this insect store glycogen, notably the so-called fat body of both developmental and adult stages. As the situation now stands, therefore, there is no substantial evidence to support a belief that amylase actually occurs within the lumen of the alimentary canal of the honeybee. There is, on the other hand, rather definite circumstantial evidence that an enzyme does occur in a place which would invalidate all the experimental work so far recorded. Since, as has been stated at length, bees starve on starch alone and fail to modify a single starch grain in the alimentary canal, there seems reason to believe that under the conditions of the experiments

recorded, the lumen of the alimentary canal does not contain amylase and therefore has not the ability to hydrolyze starch or dextrin into glucose.

It still remains to explain the presence of amylase in honey, which can hardly be doubted in view of the considerable number of investigators who have reported finding it. Since amylase is a common plant enzyme and since the components of nectar are formed in the plant by the action of enzymes, of which amylase is one, there is the possibility that amylase is present in nectars and that it still remains after the ripening process. There seems to be nothing in the process of ripening honey which would tend to destroy or weaken amylase. Auzinger (*2*) definitely states that diastase is added from the salivary glands, but offers no proof of the statement. It is a rather safe assumption that he makes this statement on the basis of previous statements that diastase occurs in the alimentary tract of the bee. No certain explanation for the presence of amylase in honey seems possible at this time. There is reason, however, to question the statement that it has its origin in the bee itself. It may not be inappropriate to point out that honeys contain dextrins after the ripening process is completed. This indicates either that diatase is absent from honey or that hydrolysis has progressed until a balance has been reached. In that event one would assume that the original nectar had contained a considerable percentage of dextrins, for which there is no evidence. It may then be that the amylase extracted from honey is derived solely from included pollen grains, which it would be impossible to remove entirely by any known process.

The absence of amylase in the lumen of the alimentary canal is significant only because of the bee's inability to digest dextrins. Bees rarely get starch with normal food, and the only normal food which might contain starch is pollen. It is often stated that pollen contains no starch (*59*) but this is quite erroneous. There is considerable variation in the starch content of pollens, the same species having starch grains in some places and not in others. Some tropical plant species have starch grains in their pollen, but as a rule starch grains are present in the pollens of plants growing at high latitudes. Various grass pollens contain starch grains, and Sturtevant (*72*) has pointed out that the presence of these minute grains may cause confusion in the diagnosis of Nosema disease of adult bees.

The inability of bees to support life on starch is interesting further in view of the fact that in the spring of the year when brood rearing is heavy and when there is at times a shortage of this material beekeepers sometimes provide their bees with substitutes for pollen (*56*). Most of the materials used, such as corn, rye, and wheat flours, are rich in starch, which doubtless serves only for the formation of feces.

INULIN

In view of the failure of honeybees to utilize starch, it was thought desirable to test their ability to maintain life with another polysaccharid of about equal complexity which requires another enzyme for its hydrolysis. Inulin is hydrolyzed by inulase but it is also readily hydrolyzed by weak acids (*21*), only 0.05 to 0.2 per cent being required at a temperature of 40° C. The acidity of the ventriculus of the honeybee in terms of hydrogen-ion concentration is P_H 6.8

to 7. Inulin belongs to the group of carbohydrates which on hydrolysis split into levulose (levulans), and the enzymes which act on them are collectively known as levulanases.

According to Straus (*71*), inulase is found in the feeding larvae of *Bombyx mori* and Hyponomenta sp., and is absent in five other species of Lepidoptera and Diptera studied. No inulase was found in the larvae of the species mentioned after they had ceased feeding, nor in the pupae or adults. Kobert (*41*) failed to find this enzyme in various invertebrates studied. For the higher animals the results obtained by various workers have apparently been in disagreement, but the more recent work has indicated that while inulase often fails to appear in the feces and while levulose does not appear in the urine of diabetic patients fed inulin, the disappearance of the inulin may be attributed to the fermentation of the inulin in the intestine without benefit to the organism (*48*, *53*, *55*). The literature on the hydrolysis of the levulasans has been well summarized by Swartz (*73*), from which it appears that with the exception of the two insects mentioned, no enzymes have been found in animals which split inulin.

It is therefore not surprising that the bees failed to be nourished by inulin fed them in the way employed in the present experiments. The bees which took this carbohydrate died as rapidly as did those without food. Pavlovsky and Zarin (*58*, *p. 526*), state that "the results obtained in our experiments allow us to conclude that the intestine of the bee does not produce inulase." In the table summarizing the results of their experiments they use a plus sign after inulase for the column headed "stomach," and a minus sign for all other parts of the alimentary tract. When the present author attempted to learn the cause of this descrepancy from the editor of the journal in which the paper by Pavlovsky and Zarin appeared he was informed that the use of the word "intestine" in the manuscript was not uniform. This word was sometimes used to designate the entire alimentary tract and sometimes to designate the intestine proper. The explanation of the descrepancy is therefore not clear.

GLYCERIN

Glycerin, a triose sugar, was tested in the series 4 feeding The bees died as soon as if starved, in fact it was not certainly determined that they took any of the glycerin. While this material is sweet to the human taste the bees were apparently not attracted by it. It has several times been suggested that glycerin be used in the making of candy for shipping queen bees by mail, since the use of glycerin would prevent the drying of the candy. This single experiment suggests that it would be unsuitable for such a purpose; nor is there any reason to suspect its availability as food. The small quantity which would be used in such candy might do no harm, but it could hardly be desirable for the bees, since it would probably cause the formation of feces in the bees at a time when such material is highly deterimental.

RHAMNOSE

While it is hardly to be expected that bees will be able to utilize pentose sugars, it nevertheless seemed interesting to test these sugars. Rhamnose (a methyl pentose) therefore was used in feeding series 5. The bees died as rapidly as if starved, although they took some of the rhamnose offered them. This sugar does not occur free in nature but is a constituent of many glucosides and of flavone derivatives.

XYLOSE

This is a pentose sugar, occurring commonly in a polysaccharide group, the pentosans, some of which have been found in honey. On this sugar (series 5) the bees died as rapidly as did those which had no food and no water. The length of life of bees fed on xylose was greater by seven times the sum of the two probable errors than that for the bees given no food but allowed water. This difference appears to have no significance.

d-ARABINOSE

This is another pentose sugar, not fermented by yeast. Bees given this sugar in feeding (series 5) died almost as rapidly as those without food and as fast as did those without food and without water.

l-ARABINOSE

Since l-Arabinose could also be obtained, it seemed interesting to determine whether the reverse molecular arrangement of this pentose sugar would have any different effect on the bees. They died when fed on this just as rapidly as did bees on the isomere.

MANNITE

This carbohydrate alcohol has been found in manna, in the sap of the larch, and in other parts of plants, and also in honeydew (*74*). It seemed therefore of more than usual interest to determine whether bees may maintain life on it. The bees in series 5 which were given this carbohydrate lived 1.318 times as long as did those without food, or 8.9 times as long as the sum of the two probable errors. This is slightly longer than the other carbohydrates of this series, which did not benefit the bees, but is in sharp contrast with the results on sucrose and trehalose. The mannite doubtless failed to benefit the bees to which it was fed.

AMYGDALIN

There was no reason to suspect that bees might be nourished by this glucoside, but it was tested to determine whether they have the ability to split it into its component parts. The material is bitter and readily soluble in water. When amygdalin is hydrolyzed by the action of the enzyme emulsin, an enzyme found in many species, it forms benzaldehyde, two molecules of glucose and hydrogen cyanide. It might be natural to assume, therefore, that if bees produce emulsin they would split this glucoside and thus release hydrogen cyanide, and that they would be poisoned thereby. If emulsin were present, it would be expected that the death rate from the splitting of amygdalin would be more rapid than from starvation. As a matter of fact, the death rate for bees fed amygdalin was practically the same as for the bees without food, which leads to the suspicion that emulsin is absent. Pavlovsky and Zarin (*58*) report the absence of emulsin with the use of amygdalin.

SALICIN

Still another glucoside, salicin, was used in series 5 with the same result as was obtained with amygdalin. This is a natural glusocide, occurring in willow bark. It is hydrolyzed by emulsin to glucose and saligenin, which in turn yields salicylic acid on oxidation. This was tested by Pavlovsky and Zarin in their tests for emulsin, with negative results. In series 5 the bees died as rapidly as did those without food.

The testing of these two gluosides was of special interest in view of the fact that it has been reported that some poisonous honeys contain andromedotoxin, a glucoside found in plants of the Ericaceae. There are numerous vague reports of honeys from this group being poisonous to man, the supposition being that the contained glucosides are the poisonous element. It may first of all be stated that poisonous honeys are of exceedingly rare occurrence, so rare in fact as to be almost nonexistent. In one case of poisonous honey from a Southern State, the evidence pointed to one of the Ericaceae as the source of the nectar. It was impossible, however, to identify pollen grains of that species in the honey. Neither was it possible chemically to detect the presence of the glucoside in the honey. In spite of these facts the honey caused distressing symptoms in those who ate it. Fortunately it had a bitter taste so that no one ate enough of it to be harmed seriously. The bees were observed working freely on this species, but there was not the slightest evidence that they were injured by the material gathered, as might have been the case if they had been able to hydrolyze the glucoside andromedotoxin, which contains hydrogen cyanide as a constituent.

COMMERCIAL GLUCOSE

This substance, sometimes called corn sirup, is a mixture of dextrin, glucose, and maltose, formed by the action of acids on starch. The same action may occur through the action of amylase. As will be seen from the data on glucose and maltose, either or both of these sugars are capable of nourishing bees, but as will be seen from the discussion of dextrin, this is not utilized for bees, forms feces in abundance, and causes activity on the part of the bees which wears them out and causes premature death. In the first feeding of commercial glucose the bees lived 2.48 times as long as did the starved bees. In the second feeding they died about as soon as the bees without food, but lived a little longer than did the bees on commercial glucose in the first feeding. What nourishment the bees received from this substance was doubtless from the glucose and maltose.

When the manufacture of commercial glucose was first begun on an extensive scale in the United States many beekeepers tried feeding it to their bees in winter. In all recorded cases dysentery developed and the losses of colonies on this food was heavy. The first difficulty encounterd was that bees did not take it readily, either because of its lack of sweetness or of the presence of some taste disagreeable to them. Bees will often starve with commercial glucose available to them. Especially in the first feeding, the amount of commercial glucose taken by the bees was smaller than for other foods on which they lived. In the second feeding, when the amounts of all the foods taken were smaller, the bees on commercial glucose died almost as rapidly as did those without food.

BROWN SUGAR

In the first feeding the bees lived considerably longer on this food than did those without food, while in the second feeding they died almost as soon as did the bees without food. Bees are obviously able to utilize sucrose which is the chief constituent of this sugar; and the fact that they died sooner on this than on chemically pure

sucrose indicates that the other constituents are less suitable. The brown sugar used was not analyzed. It was medium brown. Brown sugars contain in addition to sucrose, some reducing sugars (3 to 6 per cent), considerable ash (0.8 to 3 per cent), and moisture. There are also present certain dextrins, probably the constituents which are undesirable from the standpoint of bee feeding.

That brown sugar is totally unsatisfactory as food for bees in winter confinement was clearly shown during the period of the war when sugar was scarce. An extensive beekeeper in one of the Northern States unable to get granulated sugar, fed some light brown sugar with the result that he lost most of the colonies so fed. Since this beekeeper is highly skilled in his work, there is no reason to suspect any other cause of the death. Bees not so fed came through the winter in fine condition.

HONEYDEW HONEY

In the first feeding the bees lived almost as long on this food as they did on cane sugar or honey, and they are therefore nourished by it. The amount of dextrins present was not sufficient to cause much earlier death under the conditions of the experiment. In the second feeding the bees died almost as soon as those without food. Whether this sample of honeydew honey contained melezitose in addition to glucose, levulose, and sucrose does not appear to be essential, as bees are able to utilize all these sugars. Any detrimental effect resulting from this food doubtless came from the dextrins. The effect of honeydew honey on bees confined for a longer period in winter has been described (*63*).

HONEY

As is to be expected, the bees on this food lived as long as those on any food. In the second series the bees on levulose lived slightly longer, but the difference is well within the range of the probable errors. In the first feeding the bees on starch and cane sugar mixture lived longer than those on honey, but almost within once the probable errors. Because of the dextrin content, the honey used in these feedings would be considered of rather inferior quality for market as well as for use by bees in winter, but for the most part the bees made a good showing on it.

CONCLUSIONS

The sources of food as well as the feeding habits of the honeybee are specialized. Because of the total dissimilarity between the structure and physiology of the alimentary canal of the bee and of higher animals, analogies of function between the parts of the alimentary canal, such as the application of identical names to supposedly homologous parts, are exceedingly unwise. The foregoing experiments also show that physiological analogies between bees and higher animals are as unwarranted as are anatomical ones. Even in closely related insects, where there are closely homologous structures in the alimentary canal, it is unwise to assume the same abilities to absorb and assimilate certain materials as food, because of homologous structure and apparent physiological homologies. Because of the great diversity of foods used, enzymes of all kinds may be assumed to exist among insects. In some instances the enzymes have been

found, but this is no basis for assuming that they will also occur in related species. Because of the diversity of feeding habit, it seems necessary to determine the availability of various materials for almost every species separately and to avoid guesses from analogy.

In the present studies, only the carbohydrates have been investigated for their availability as food for bees. This is possible by the method used, because bees frequently remain alive and active for a considerable time with no food other than carbohydrates.

Of the monohexose sugars, bees utilize as food glucose and levulose, but not galactose and probably not mannose. Of the disaccharides they utilize sucrose, trehalose, and maltose but not lactose. Of the trisaccharides they utilize melezitose but not raffinose. They fail to utilize as food the more complex polysaccharides dextrin, starch, or inulin. It is evident from the fact that they do not die more rapidly when given the glucosides containing hydrogen cyanide as a constituent that bees lack the enzyme emulsin. They do not utilize glycerin, mannite, or the four pentose sugars, rhamnose, xylose, *d*-Arabinose, and *l*-Arabinose. They can live on commercial glucose for a time from the dextrose and maltose present, but show a pronounced dislike for this material, and it does not serve as a safe food. They can live on brown sugar, but practical experience in the apiary shows its unsuitability for winter feed. Honeydew contains sugar which bees utilize but it is unsafe for winter. Honey, even the rather inferior type used in this experiment, is as good as any food material for bees, and there is reason to believe, from data not herein contained, that it is far better for bees at certain times of year than any artificial sugar obtainable.

Interpreting these findings in terms of enzymes, it appears that the honeybee contains invertase, maltase, and trehalase but not lactase, emulsin, inulose, amylase (diastase), and dextrase, if this is to be separated from amylase. No evidence is herein contained on enzymes other than those which are concerned in the hydrolizing of carbohydrates.

From the point of view of practical beekeeping, the absence of enzymes of the amylase-dextrase group is of first importance, and the work here recorded supports the theory that dysentery in winter is caused by the indigestibility of dextrins in the winter food supply, and the consequent accumulation of this material in the rectal ampulla as fecal material.

LITERATURE CITED

(1) Armstrong, E. F.
1919. The simple carbohydrates and the glucosides. Ed. 3, 239 p. London, New York [etc.].

(2) Auzinger, A.
1910. Über fermente im honig und den wert ihres nachweises für die honigbeurteilung. Ztschr. Untersuch. Nahr. u. Genussmtl. 19: [65]–83.

(3) ———
1910. Weitere beiträge zur kenntnis der fermentreaktionen des honigs. Ztschr. Untersuch. Nahr. u. Genussmtl. 19: [353]–362.

(4) Axenfeld, D.
1903. Invertin im honig und im insektendarm. Zentbl. Physiol. 17: 268–269.

(5) Bachmann, F. M.
1919. Vitamine requirements of certain yeasts. Jour. Biol. Chem. 39: 235–257, illus.

(6) Bayliss, W. M.
1919. the nature of enzyme action. Ed. 4, 190 p., illus. London, New York [etc.].
(7) Berthelot, M.
1857. sur diverses matières sucrées. Compt. Rend. Soc. Biol. [Paris] (2) 4: 112–115.
(8) ———
1858. sur le tréhalose, nouvelle espèce de sucre. Compt. Rend. Acad. Sci. [Paris] 46: 1276–1279.
(9) ———
1859. nouvelles recherches sur les corps analogues au sucre de canne. Ann. Chim. et Phys. (3) 55: 269–296, illus.
(10) Bertholf, L. M.
1927. the utilization of carbohydrates as food by honeybee larvae. Jour. Agr. Research 35: 429–452, illus.
(11) Biedermann, W.
1910. die aufnahme, verarbeitung und assimilation der nahrung. neunter teil. die ernährung der insekten (hexapoda). *In* Winterstein, H., Handbuch der vergleichenden Physiologie, Bd. 2, p. 726–902, illus. Jena.
(12) Bierry, H.
1909. dédoublement diastasique des α et β-méthyl-d-glucosides. Compt. Rend. Acad. Sci. [Paris] 149: 314–316.
(13) Bonnier, G.
1878. les nectaires. étude critique, anatomique et physiologique. Ann. Sci. Nat., Bot. (6) 8: 5–212, illus.
(14) ——— and Flahault, C.
1878. observations sur les modifications des végétaux suivant les conditions physiques du milieu. Ann. Sci. Nat., Bot. (6) 7: [93]–125.
(15) Bourquelot, E., and Gley, E.
1895. note concernant l'action du sérum sanguin et de l'urine sur le tréhalose. Compt. Rend. Soc. Biol. [Paris] (10) 2: 515–516.
(16) ——— and Gley, E.
1895. digestion du tréhalose. Compt. Rend. Soc. Biol. [Paris] (10) 2: 555–557.
(17) Browne, C. A.
1908. chemical analysis and composition of american honeys. U. S. Dept. Agr., Bur. Chem. Bul. 110, 93 p., illus.
(18) ———
1912. a handbook of sugar analysis. 787 p., illus. New York and London.
(19) Büsgen, M.
1891. der honigtau. biologische studien an pflanzen und pflanzenläusen. Jena. Ztschr. Naturw. (n. F. 18) 25: 339–428, illus.
(20) Caillas, A.
1926. le miel contient des vitamines. i–ii. France Apic. 32: 3–5, 54–55.
(21) Chittenden, R. H.
[1899]. the behavior of inulin in the gastro-intestinal tract. Amer. Jour. Physiol. 2: XVII–XVIII. (Proc. Amer. Physiol. Soc. 11, 1898.)
(22) Dutcher, R. A.
1918. vitamine studies. iii. observations on the curative properties of honey, nectar, and corn pollen in avian polyneuritis. Jour. Biol. Chem. 36: 551–555.
(23) Erlenmeyer, E.
1874. ueber die fermente in den bienen, im bienenbrot und im pollen und über einige bestandtheile des honigs. Sitzber. K. Akad. Wiss. München, Math. Phys. Kl. 4: 204–207.
(24) ———
1880. bemerkungen zu vorstehender mittheilungen. [chemische studien über die thätigkeit der bienen.] Bienen Ztg. 36: 3–4.

(25) Erlenmeyer, E., and Planta-Reichenau, A. von
1878–80. chemische studien über die thätigkeit der bienen. Bienen Ztg. 34: 181–183, 1878; 35: 2–6, 155–158, 1879; 36: 2–3, 1880.
(26) Faber, H. K.
1920. a study of the antiscorbutic value of honey. Jour. Biol. Chem. 43:113–116.
(27) Fellenberg, T. von.
1911. ueber invertase und diastase im honig. Mitt. Lebensmtl. u. Hyg., Eidg. Gsndhtsamt. [Switz.] 2: [369]–377.
(28) Fischer, E.
1895. ueber den einfluss der configuration auf die wirkung der enzyme. iii. Ber. Deut. Chem. Gesell. 28: 1429–1438.
(29) ——— and Delbrück, K.
1909. synthese neuer disaccharide vom typus der trehalose. Ber. Deut. Chem. Gesell. 42: 2776–2785.
(30) Gothe, F.
1914. die fermente des honigs. Ztschr. Untersuch. Nahr. u. Genussmtl. 28: [273]–286, illus.
(31) Green, J. R.
1895. researches on the germination of the pollen grain and the nutrition of the pollen tube. Roy. Soc. [London], Phil. Trans. (B) 185: 385–409.
(32) Guibourt.
1858. notice sur le tréhala. Jour. Pharm. et Chim. (3) 34: 81–87.
(33) ———
1858. notice sur une matière pharmaceutique nommée le tréhala, produite par un insecte de la famille des charançons. Compt. Rend. Acad. Sci. [Paris] 46: 1213–1217.
(34) Hawk, P. B., Smith, C. A., and Bergeim, O.
1921. the vitamine content of honey and honey comb. Amer. Jour. Physiol. 55: 339–348, illus.
(35) Heddon, J.
1885. success in bee-culture as practiced and advised. 128 p., illus. Dowagiac, Mich.
(36) Hudson, C. S., and Paine, H. S.
1910. the inversion of cane sugar by invertase. iv. the influence of acids and alkalies on the activity of invertase. Jour. Amer. Chem. Soc. 32: 774–779, illus.
(37) ——— and Sherwood, S. F.
1920. the occurrence of melezitose in honey. Jour. Amer. Chem. Soc. 42: 116–125.
(38) Jost, L.
1907. lectures on plant physiology. Authorized English translation by R. J. H. Gibson. 564 p., illus. Oxford.
(39) Kastle, J. H., and Clark, M. E.
1903. on the occurrence of invertase in plants. Amer. Chem. Jour. 30: [422]–427.
(40) Kjeldahl, J.
1879. recherches sur les ferments producteurs de sucre. Meddel. Carlsberg Lab. 1: [107]–184, illus. [In Danish. French résumé, p. 109–157. Separately paged.]
(41) Kobert, R.
1903. ueber einige enzyme wirbelloser thiere. Pflüger's Arch. Physiol. 99: 116–186.
(42) Kœhler, A.
1921–22. l'abeille présente-t-elle dans son corps des matières de réserve pour le repos hivernal? Bul. Soc. Romande Apicult. 18: 264–267, 1921; 19: 14–15, 1922.
(43) Kondo, K.
1912. über synthetische aminosäurebildung in der leber. iii. mitteilung. die bildung körperfremder aminosäuren. Biochem. Ztschr. 38: [407]–413.
(44) Langer, J.
1902. zur beurtheilung des honigs mittels der in ihm nachweisbaren fermente. (Abstract) Ztschr. Untersuch. Nahr. u. Genussmtl. 5: 1204–1205.

(45) LANGER, J.
1903. FERMENTE IM BIENENHONIG. Schweiz. Wchnschr. Chem. u. Pharm. 41: 17–18.
(46) ———.
1909. BEURTEILUNG DES BIENENHONIGS UND SEINER VERFÄLSCHUNGEN MITTELS BIOLOGISCHER EIWEISSDIFFERENZIERUNG. Arch. Hyg. 71: 308–330.
(47) LENZ, W.
1910. EIN NEUES PEPTISCHES ENZYM AUS HONIG. Apoth. Ztg. 25: 678–679.
(48) LEWIS, H. B.
1912. THE VALUE OF INULIN AS A FOODSTUFF. Jour. Amer. Med. Assoc. 58: 1176–1177.
(49) MCINDOO, N. E.
1914. THE OLFACTORY SENSE OF THE HONEY BEE. Jour. Expt. Zool. 16: 265–346, illus.
(50) MAQUENNE, L.
1891. RECHERCHES SUR LA TRÉHALOSE. Compt. Rend. Acad. Sci. [Paris] 112: 947–950.
(51) ———.
1899. SUR LA MIELLÉE DE L'EVONYMUS JAPONICA. Bul. Soc. Chim. Paris (3) 21: 1082–1083.
(52) MARPMANN, G.
1903. BEITRÄGE ZUR PRÜFUNG UND BEURTEILUNG DES BIENENHONIGS. Pharm. Ztg. 48: 1010.
(53) MENDEL, L. B., and MITCHELL, P. H.
1905. ON THE UTILIZATION OF VARIOUS CARBOHYDRATES WITHOUT INTERVENTION OF THE ALIMENTARY DIGESTIVE PROCESSES. Amer. Jour. Physiol. 14: 239–247.
(54) MITSCHERLICH.
1858. UEBER DIE MYCOSE, DEN ZUCKER DES MUTTERKORNS. Jour. Prakt. Chem. 73: 65–70, illus.
(55) MIURA, K.
1895. WIRD DURCH ZUFUHR VON INULIN BEIM PFLANZENFRESSER DIE GLYKOGENBILDUNG IN DER LEBER GESTEIGERT? Ztschr. Biol. (n. F. 14) 32: [255]–265.
(56) PARKER, R. L.
1926. THE COLLECTION AND UTILIZATION OF POLLEN BY THE HONEYBEE. N. Y. Cornell Agr. Expt. Sta. Mem. 98, 55 p., illus.
(57) PATON, J. B.
1921. POLLEN AND POLLEN ENZYMES. Amer. Jour. Bot. 8: 471–501.
(58) PAVLOVSKY, E. N., and ZARIN, E. J.
1922. ON THE STRUCTURE OF THE ALIMENTARY CANAL AND ITS FERMENTS IN THE BEE (APIS MELLIFERA L.). Quart. Jour. Micros. Sci. [London] (n. s. no. 263) 66: [509]–556, illus.
(59) PETERSEN, H.
1912. BEITRÄGE ZUR VERGLEICHENDEN PHYSIOLOGIE DER VERDAUUNG. V. DIE VERDAUUNG DER HONIGBIENE. Pflüger's Arch. Physiol. 145: 121–151, illus.
(60) PHILLIPS, E. F.
1922. THE EFFECT OF ACTIVITY ON THE LENGTH OF LIFE OF HONEYBEES. Jour. Econ. Ent. 15: 368–371.
(61) ———
1922. THE ABILITY OF QUEEN AND DRONE HONEYBEES TO FEED THEMSELVES. Jour. Econ. Ent. 15: 430–432.
(62) ———
1924. THE DIGESTION OF THE HONEYBEE. Gleanings Bee Cult. 52: 76–79.
(63) ——— and DEMUTH, G. S.
1914. THE TEMPERATURE OF THE HONEYBEE CLUSTER IN WINTER. U. S. Dept. Agr. Bul. 93, 16 p., illus.
(64) PIERCE, W. D.
1915. THE USES OF CERTAIN WEEVILS AND WEEVIL PRODUCTS IN FOOD AND MEDICINE. Ent. Soc. Wash. Proc. 17: 151–154, illus.
(65) PIRSCH, G. B.
1923. STUDIES ON THE TEMPERATURE OF INDIVIDUAL INSECTS, WITH SPECIAL REFERENCE TO THE HONEY BEE. Jour. Agr. Research 24: 275–288, illus.

(66) Planta-Reichenau, A. von
1875. ueber die fermente in den bienen, im bienenbrot und im pollen und über einige bestandtheile des honigs. Verhandl. Schweiz. Naturf. Gesell. Chur (1873/74) 57: 101–104.
(67) ———
1888. ueber den futtersaft der bienen. Hoppe-Seyler's Ztschr. Physiol. Chem. 12: [327]–354.
(68) ———
1892. ueber honigbildung. Jahresber. Naturf. Gesell. Graubünden (n. F.) 35: 140–148.
(69) Scheunert, A., Schieblich, M., and Schwanebeck, E.
1923. zur kenntnis der vitamine. I. mitteilung. über den vitamingehalt des honigs. Biochem. Ztschr. 139: [47]–56.
(70) Starling, E. H.
1906. mercers' company lectures on recent advances in the physiology of digestion. 156 p., illus. Chicago.
(71) Straus, J.
1908. über das vorkommen einiger kohlehydratfermente bei lepidopteren und dipteren in verschiedenen entwicklungsstadien. Ztschr. Biol. (n. F. 34) 52: [95]–106.
(72) Sturtevant, A. P.
1919. a source of confusion in the diagnosis of nosema apis in adult bees. Jour. Econ. Ent. 12: 269–270.
(73) Swartz, M. D.
1911. nutrition investigations on the carbohydrates of lichens, algae, and related substances. Conn. Acad. Arts and Sci. Trans. 16: 247–382.
(74) Unger, F.
1857. beiträge zur physiologie der pflanzen. v. zur näheren kenntniss des honigthaues. Sitzber. K. Akad. Wiss. Wien, Math. Naturw. Kl. 25: 449–459.
(75) Utz.
1908. ueber die marpmannsche reaktion zur unterscheidung von schleuderhonig und von honig, der durch erhitzen gewonnen wurde. Ztschr. Öffentl. Chem. 14: [21]–28.
(76) White, P. B.
1921. the normal bacterial flora of the bee. Jour. Path. and Bact. 24: 64–78, illus.
(77) Wiggers, H. A. L.
1832. untersuchung über das mutterkorn, secale cornutum. Ann. Pharm. 1: [129]–182.
(78) Wilson, A. S.
1878. on the amounts of sugar contained in the nectar of various flowers. Chem. News 38: 93.
(79) Winterstein, E.
1893. zur kenntniss der trehalose. Ber. Deut. Chem. Gesell. 26: 3094–3098.
(80) ———
1894. zur kenntniss der trehalose. Ztschr. Physiol. Chem. 19: 70–83.
(81) Zarin, E.
1921. beiträge zur chemie der bildung und reifung des bienenhonigs. Acta Univ. Latviensis 1.
(82) ——— (Sarin, E.).
1923. über fermente der verdauungsorgane der honigbiene. Biochem. Ztschr. 135: [59]–74.
(83) ———
1923. weitere studien über invertase des darmkanals der honigbiene. Biochem. Ztschr. 135: [75]–84.

THE UTILIZATION OF CARBOHYDRATES AS FOOD BY HONEYBEE LARVAE[1]

By L. M. BERTHOLF[2]

Formerly Junior Biologist in Apiculture, Bee Culture Investigations, Bureau of Entomology, United States Department of Agriculture[3]

INTRODUCTION

The food of honeybee larvae, as of animals in general, consists of mixtures of carbohydrates, proteins, fats, mineral salts, water, and presumably vitamins. But, of course, this statement does not mean that the larvae can utilize all the carbohydrates, all the proteins, all the fats, etc. Thus far, however, no one has reported any attempt to discover which particular ones from each of these classes of foods the larvae can use.

Since carbohydrates constitute one of the most important classes of foods, and since they are for the most part well known chemically, it has seemed to the writer logical to begin the consideration of this problem with an investigation of the ability of the larvae to use carbohydrates as food.

This investigation seemed worth while for two reasons: (1) It might be of practical value to the beekeeper to know which carbohydrates the larvae can utilize, since during the latter part of their larval life workers and drones are fed large quantities of carbohydrates, often furnished by the beekeeper; (2) from the point of view of comparative physiology it would be of interest to know how honeybee larvae compare with honeybee adults, and also with other insect larvae and adults, in their ability to utilize certain carbohydrates.

REVIEW OF PREVIOUS WORK ON DIGESTION OF CARBOHYDRATES IN INSECTS

A review of the literature regarding carbohydrate digestion in insects reveals, as mentioned above, no previous work on honeybee larvae, but shows considerable work on honeybee adults and on larvae and adults of other insects in several of the orders. (Table 1.) Most of the work has been a search for enzymes in the various portions of the alimentary tract. Usually an extract was made of the organ under investigation and the action of this extract was tested on various carbohydrates. According to this method, if a carbohydrate was hydrolyzed by the extract, the enzyme peculiar to that process was supposed to be present.

In a few instances, in the investigations of Cleveland (*7*),[4] Sieber and Metalnikow (*21*), Chapman (*6*), and Phillips (*17*), a direct feeding

[1] Received for publication May 19, 1927; issued October, 1927. This paper, prepared in part fulfillment of the requirements for the degree of master of arts at Johns Hopkins University, is a joint contribution from the Bureau of Entomology, U. S. Department of Agriculture, and Johns Hopkins University. The experimental work was done at the bee culture laboratory of the Bureau of Entomology, at Somerset, Md.

[2] The writer thanks E. F. Phillips, formerly senior apiculturist; the present members of the staff of the bee culture laboratory; and S. O. Mast, of Johns Hopkins University, for valuable suggestions and assistance during the course of the investigations herein reported.

[3] Now professor of biology, Western Maryland College, Westminster, Md.

[4] Reference is made by number (italic) to "Literature cited," p. 451.

method was employed, a group of insects being fed on a certain carbohydrate until all of its members died and the length of life of such a group being compared to that of other groups kept as checks. If the group fed on a certain carbohydrate lived longer than a similar, starved group, its members were supposed to possess the ability to utilize that carbohydrate as food.

TABLE 1.—*A summary of investigations on the digestion of carbohydrates by insects*

Name of insect	Part investigated [a]	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Orthoptera:												
Blatta orientalis.	Extract of salivary gland.	Basch (*2*)	1858	+								
Do	Extract of walls of mid-gut.	do	1858	+								
Do	Secretion of salivary glands.	J. de Belesme (*11*).	1876	+								
Do	Secretion of gastric coeca	do	1876	−								
Do	Secretion of Malpighian tubes.	do	1876	−								
Do	Extract of salivary glands.	Swingle (*24*)	1925	+	−	−	−					
Do	Extract of fore-gut	do	1925	−	+	+	−					
Do	Extract of gastric coeca	do	1925	−	+	+	−					
Do	Extract of mid-gut	do	1925	−	+	+	−					
Do	Extract of hind-gut	do	1925	−	−	−	−					
Periplaneta americana.	Pulverized salivary glands.	Plateau (*19*)	1876	+								
Stetheophyma grossum.	Extract of salivary glands.	Plateau (*18*)	1874	+								
Locusta viridissima.	do	do	1874	+								
Do	Extract of salivary glands, pupa.	do	1874	+								
Do	Extract of salivary glands, larva.	do	1874	+								
Isoptera:												
Reticulitermes flavipes.	Ability to live	Cleveland (*7*).	1924					−				
Hemiptera:												
Cicada sp	Extract of whole gut	Axenfeld (*1*)	1904		+							
Aphrophora salicis (De Geer).	Extract of salivary glands, larva.	Gruner (*10*)	1900	+								
Do	Extract of alimentary tract, larva.	do	1900	−								
Nepa cinerea	Extract of salivary glands.	Plateau (*18*)	1874	+								
Ranatra linearis.	do	do	1874	+								
Notonectes sp.	Extract of whole gut	Axenfeld (*1*)	1904		+							
Lepidoptera:												
Liparis dispar.	Secretion of mid-gut of larva.	Plateau (*18*)	1874	+								
Pieris brassicae.	Extract of gut of larva	Biedermann (*4*).	1910	+								
Do	Extract of gut, adult	Axenfeld (*1*)	1904		+							
Tineola biselliella.	Excrement of larva	Sitowski (*22*).	1905	(b)				−				
Galleria mellonella.	Ability to live	Sieber and Metalnikow (*21*).	1904	−	−							
Do	Extract of intestine of larva.	do	1904	+								
Do	Extract of whole feeding larva.	Straus (*23*)	1909	+	+				−	+		

[a] The adult insect is referred to unless otherwise stated.

[b] Very little.

TABLE 1.—*A summary of investigations on the digestion of carbohydrates by insects*—Continued

Name of insect	Part investigated	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Lepidoptera—Continued.												
Euproctis chrysorrhoea.	Extract of whole feeding larva.	Straus (*23*)	1909	+	+[c]	+	+[d]	....	?[e]	+	....	....
Do	Extract of whole pupa	do	1909	+	+	....	+?	....	?	+	....	....
Ocneria dispar.	Extract of whole feeding larva.	do	1909	+	+	+	−	....	?	+	....	(*f*)
Bombyx neustria.	do	do	1909	+	+[e]	....	−	....	−	+	....	....
Do	Extract of whole pupa	do	1909	−?	+	+	−	....	−	+	....	....
Bombyx mori.	Analysis of food and excrement.	Kellner (*12*)	1884	....	....	....	....	−	....	....	....	....
Do	Extract of whole feeding larva.	Straus (*23*)	1909	+	+	....	+	....	+	+	....	....
Do	Extract of whole not-feeding larva.	do	1909	(*e*)	+	....	−	....	−	−	....	....
Do	Extract of intestine of larva.	Axenfeld (*1*)	1904	−	−	....	....	....	....	....	+	....
Do	Extract of whole pupa, 10 days.	Straus (*23*)	1909	+	+	+?	−	....	−	+	....	....
Do	Extract of whole pupa, 14 days.	do	1909	+	+	(*e*)	−	....	−	+	....	....
Do	Extract of whole pupa, 18 days.	do	1909	−	+	−	−	....	−	+	....	....
Do	Extract of whole pupa, 21 days.	do	1909	−[h]	....	....	....	....	....	....	....	....
Do	Extract of whole adult	do	1909	{−[i] +[h]}	+	....	−	....	−	....	....	....
Hyponomeuta sp.	Extract of whole feeding larva.	do	1909	+	+	+	+	....	+	+	....	....
Do	Extract of whole pupa	do	1909	+	+	?	−	....	+	+	....	....
Carpocapsa pomonella.	Extract of gut of larva	Axenfeld (*1*)	1904	....	+	....	....	....	....	....	....	....
Vanessa sp.	Extract of gut	do	1904	....	+	....	....	....	....	....	....	....
Vanessa urticae.	Extract of whole pupa	Straus (*23*)	1909	....	....	+	−	....	....	....	....	....
Diptera:												
Calliphora vomitoria.	Extract of whole feeding larva.	do	1909	+	+	+	−	....	−	+?	....	....
Do	Extract of whole not-feeding larva.	do	1909	+	+	−?	−	....	−	+	....	....
Do	Extract of whole pupa	do	1909	+	+	+	−	....	−	+	....	....
Do	Extract of whole adult	do	1909	+	+	+	−	....	−	+	....	....
Do	Extract of excrement	Weinland (*25*).	1906	−	....	....	....	....	....	....	....	....
Do	Extract of whole adult	Weinland (*26*).	1907	+	....	....	....	....	....	....	....	....
(Musca) Sarcophaga carnaria.	Extract of gut of larva	Axenfeld (*1*)	1904	....	−	....	....	....	....	....	....	....
Do	Extract of gut of adult	do	1904	....	+	....	....	....	....	....	....	....
Musca domestica.	Extract of whole adult	Kobert (*13*)	1903	+	....	....	....	....	−	....	−	....
Coleoptera:												
May beetles	do	do	1903	+	....	....	....	....	....	....	....	....
Tenebrio molitor.	Contents of mid-gut of larva.	Frenzel (*9*)	1882	+	....	....	....	....	....	....	....	....
Do	Extract of mid-gut of larva.	Biedermann (*3*).	1898	+	+	+	....	−	....	....	....	....
Carabus sp.	Extract of whole gut	Axenfeld (*1*)	1904	....	(*i*)	....	....	....	....	....	....	....
Dytiscus dimidiatus.	Fluid in crop of adult	Plateau (*18*)	1874	−	....	....	....	....	....	....	....	....
Do	Extract of mid-gut	do	1874	−	....	....	....	....	....	....	....	....
Do	Extract of small intestines.	do	1874	−	....	....	....	....	....	....	....	....

[c] Both in gut and in body.
[d] In the gut; questionably, in the body.
[e] Question marks in these columns mean doubtfully positive results.
[f] No agar ferment.
[g] Little.
[h] For glycogen diastase.
[i] For starch diastase.
[j] Weak.

TABLE 1.—*A summary of investigations on the digestion of carbohydrates by insects*—Continued

Name of insect	Part investigated	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Coleoptera—Con.												
Dytiscus sp.	Extract of whole gut	Axenfeld (*1*)	1904	....	(*f*)	....	....	....	....	....	....	....
Dytiscus marginalis.	Fluid in crop of adult	Plateau (*18*)	1874	−	....	....	....	....	....	....	....	....
Phymatodes variabilis.	Extract of mid-gut of larva.	Seillière(*20*)	1905	....	....	....	....	....	....	....	....	(*h*)
Tribolium confusum.	Ability to live	Chapman (*6*).	1924	+	....	....	....	....	....	....	....	....
Oryctes nasicornis.	Extract of mid-gut of adult.	Plateau (*18*)	1874	+	....	....	....	....	....	....	....	....
Melolontha vulgaris.	do	do	1874	+	....	....	....	....	....	....	....	....
Melolontha sp.	Extract of intestine	Axenfeld(*1*)	1904	....	(*f*)	....	....	....	....	....	....	....
Hydrous carboides.	Fluid in oesophagus	Plateau(*18*)	1874	+	....	....	....	....	....	....	....	....
Do	Contents of mid-gut	do	1874	+	....	....	....	−	....	....	....	....
Do	Extract of mid-gut wall	do	1874	+	....	....	....	....	....	....	....	....
Hydrophilus piceus.	Fluid in oesophagus	do	1874	+	....	....	....	....	....	....	....	....
Do	Contents of mid-gut	do	1874	+	....	....	....	−	....	....	....	....
Do	Extract of mid-gut wall	do	1874	+	....	....	....	....	....	....	....	....
Hydrophilus sp.	Extract of intestine	Axenfeld (*1*)	1904	....	+	....	....	....	....	....	....	....
Hymenoptera:												
Ants	Extract of whole pupa	Kobert (*13*)	1903	+	....	....	....	....	....	....	....	(*i*)
Apis mellifica	Extract of head	Erlenmeyer (*8*).	1874	+	+	....	....	....	....	....	....	....
Do	Extract of thorax	do	1874	(*i*)	(*i*)	....	....	....	....	....	....	....
Do	Extract of abdomen	do	1874	+	+	....	....	....	....	....	....	....
Do	Extract of fore-gut	Axenfeld (*1*)	1904	....	+	....	....	....	....	....	....	....
Do	Extract of mid-gut and of hind-gut.	do	1904	....	(*i*)	....	....	....	....	....	....	....
Do	Known substance fed; analyzed in mid-gut.	Petersen (*16*)	1912	(*i*)	+	....	....	−	....	....	....	....
Do	Known substance fed; analyzed in fore-gut.	do	1912	−	+	....	....	....	....	....	....	....
Do	Extract of mid-gut	do	1912	+	....	....	....	....	....	....	....	....
Do	Extract of fore-gut	Pavlovsky and Zarin (*15*).	1922	−	−	....	−	....	−	....	−	....
Do	Extract of ventriculus	do	1922	+	+	....	−	....	−	....	−	....
Do	Extract of small intestine	do	1922	−	−	....	−	....	−	....	−	....
Do	Extract of large intestine	do	1922	−	−	....	−	....	−	....	−	....
Do	Ability to live	Phillips (*17*)	1924	−	+	+	−	....	−	....	−	(*m*)

f Weak. *h* Xylanase. *i* Zymase. *m* Melezitase; trehalase.

For the purpose of comparison, the results of the various investigators are brought together and presented in Table 1. This table gives the name of the insect, the part of the insect investigated, the method, the name of the investigator, the year, and a list of all the carbohydrate-digesting enzymes found. The insects are listed under their respective orders, but no attempt is made to classify the insects within an order.

Regarding the use and scope of Table 1 attention should be called to the following facts:

1. The table merely indicates in any case that the enzyme was, or was not, found, and does not usually indicate its quantity or intensity.

2. Negative results mean simply that the investigator tested for the enzyme in question and did not find it. It may be that the enzyme was present in quantities too small to be detected by the test used.

3. In the case of feeding experiments it is assumed that the ability to use a substance as food means that the enzyme necessary to digest that food is present.

4. A host of insects whose feeding habits are well known in a general way, but whose digestive abilities have not been specifically investigated, are for obvious reasons excluded from the table.

One may briefly summarize as follows the results of previous investigations regarding the ability of insects in general to digest carbohydrates:

1. Amylase is very widely distributed among insects, having been demonstrated in nearly every species investigated, but is often localized in a particular organ.

2. In but very few species has the insect been found entirely lacking in any of the common carbohydrate-digesting enzymes, i. e., amylase, invertase, and maltase.

3. No insect investigated has been shown to be able to digest cellulose. It is quite probable, however, that many insects not investigated can do so.

4. In the case of most species every enzyme present is of direct use in digesting the food which the insect eats.

5. With some few species, however, enzymes occur which are probably never used, e. g., amylase in ant pupae and trehalase in honeybees.

6. In the case of a few other species substances are habitually eaten for which the insect secretes no digesting enzymes, e. g., wood by termites.

In regard to honeybees in particular, the review of the literature shows the following facts (see Table 1, *Apis mellifica*):

1. All work of this sort has been done on adults; none on the larvae.

2. All work except Phillips's and part of Petersen's has been done by the method of extracts.

3. All investigators using the method of extracts have found amylase somewhere in the animal, yet Phillips found no ability to utilize starch as food.

4. All investigations have revealed invertase somewhere in the animal.

5. In two investigations lactase, inulase, and emulsin were sought, either directly or indirectly, but were not found.

6. From one investigation it appears that cellulase is not present.

7. From one investigation it appears that bees can readily utilize the rare sugars, melezitose and trehalose.

The problem now is to ascertain how the larvae of honeybees compare with adult honeybees and with other insects in their ability to utilize carbohydrates as food.

MATERIAL AND METHOD

Two methods of investigation were open in approaching this problem, the method of making extracts of various organs and testing the action of these extracts on various carbohydrates, and the method of feeding a group of larvae on a given carbohydrate and ascertaining whether or not the larvae receive any nourishment from it, by comparing the length of life of this group with that of a similar group fed on water only.

67589—27——4

The latter method, the direct method, was chosen for two reasons: (1) It enabled one to test any number of carbohydrates in a direct manner, without encountering the technical errors present in many of the chemical tests for carbohydrates; in fact, there are as yet no specific chemical tests for many carbohydrates; (2) the results discovered by direct feeding would seem to be of more practical value than results obtained by the method of extracts, since in the former only those enzymes are considered which are of actual use in keeping the larva alive.

In performing an experiment to compare the length of life on a given food with the length of life on water alone the procedure was as follows: Enough larvae of as uniform size as possible were removed from the comb to serve for the whole experiment. As each larva was taken from the comb with a transferring needle it was floated out on water, where any food clinging to it was washed off. After a sufficient number of larvae had been removed they were divided into lots of 10 to 25 each, and each lot was weighed. The weight was usually about 18 mgm. per larva, although in some experiments the average was as low as 11 mgm. and in others as high as 46 mgm. According to Nelson and Sturtevant (*14*), the weight of larvae at hatching averages 0.100 mgm.; at 1 day of age, 0.650 mgm.; at 2 days, 4.745 mgm.; at 3 days, 24.626 mgm.; and at 4 days, 93.990 mgm.; it follows that larvae weighing 18 mgm. are, on an average, nearly 3 days old.

After weighing, the larvae were transferred to a small watch glass containing a solution of the carbohydrate to be tested; the watch glass was placed inside a Petri dish containing a little water, to serve as a moist chamber; and the whole was put inside an incubator at about hive temperature (35° C.). An experiment consisted of a number of such lots of similar larvae, fed on various concentrations of the food in question, together with one lot supplied with water only, as a check. For most of the lots examinations were made at intervals of six hours, the dead being removed at this time and the food replaced by a fresh supply if it appeared in the least cloudy. A larva was considered dead if it no longer responded by muscular contraction when touched with the transferring needle. The time at which death occurred was assumed to have been midway between the time of the examination at which it was found dead and the time of the last previous examination. The carbohydrates used were dextrose, levulose, galactose, sucrose, maltose, lactose, trehalose, melezitose, starch, glycogen, and dextrin, in various concentrations, and, finally, a series of honeys. Lots were run in groups of 5 to 10 at a time, and a starvation check, in which distilled water alone was fed, was run with each group. The expressions "starvation check" and "water check" are used interchangeably throughout this paper. Often two or three such groups or series were run for a single carbohydrate.

The complete record of a typical lot, as given in Table 2, will make clear the procedure in each case.

Other lots fed at the same time and under similar conditions had, as average lengths of life, those fed on 5 per cent sucrose, 11.80 ± 0.62 hours; 15 per cent sucrose, 17.98 ± 1.18 hours; 25 per cent sucrose, 32.90 ± 2.95 hours; 50 per cent sucrose, 59.66 ± 4.23 hours; and water, 8.68 ± 0.15 hours.

TABLE 2.—*A complete record of all the observations and computations made for a typical lot of honeybee larvae* [a]

[Lot 83; food, 35 per cent sucrose; [b] weight, 0.0185 gm. each]

Date, 1924	Hour	Number of larvae alive	Number of larvae died since last examination (=n)	Length of life (hours) (=l)	n×l
Aug. 18	12 m.	20			
Do	6 p. m.	19	1	3	3
Do	11.30 p. m.	19			
Aug. 19	6 a. m.	19			
Do	12 m.	18	1	21	21
Do	6 p. m.	18			
Do	11.30 p. m.	18			
Aug. 20	6 a. m.	18			
Do	12 m.	15	3	45	135
Do	6 p. m.	11	4	51	204
Do	11.30 p. m.	6	5	57	285
Aug. 21	6 a. m.	4	2	63	126
Do	12 m.	2	2	69	138
Do	6 p. m.	2			
Do	12 p. m.	1	1	81	81
Aug. 22	6 a. m.	0	1	87	87
Total			20		1,080

[a] All other lots in these experiments were treated in a similar way.
[b] Thirty-five per cent sucrose means 35 gm. of pure sucrose crystals in 100 c. c. of solution. All solutions mentioned were made in the same way.

Mean length of life, 54 hours.
Probable error of the mean (Peters' formula), 2.39 hours.

It was possible in this way to get a comparison of the effect of any concentration of any carbohydrate desired on the length of life of the larvae.

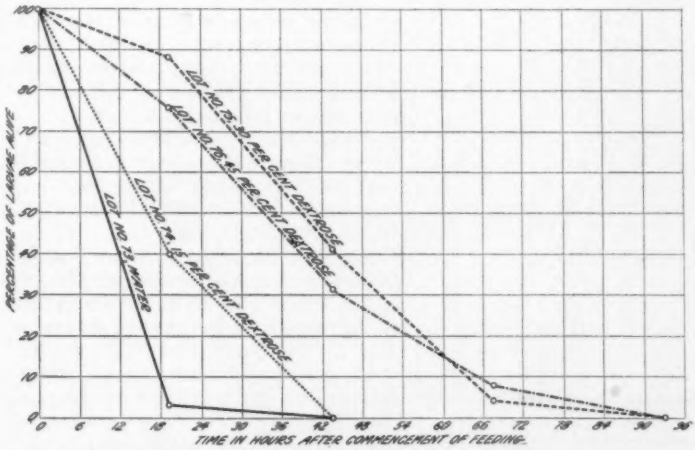

PERCENTAGE OF LARVAE ALIVE
LOT NO 75, 20 PER CENT DEXTROSE
LOT NO 76, 45 PER CENT DEXTROSE
LOT NO 74, 15 PER CENT DEXTROSE
LOT NO 73, WATER
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING

FIG. 1.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of dextrose in certain concentrations with water, and of one lot supplied with water only

The data obtained in these feeding experiments are presented in Tables 3 to 22 and Figures 1 to 11. The experimental results are

given under the headings monosaccharides, disaccharides, trisaccharides, polysaccharides, and honeys. Following these a section

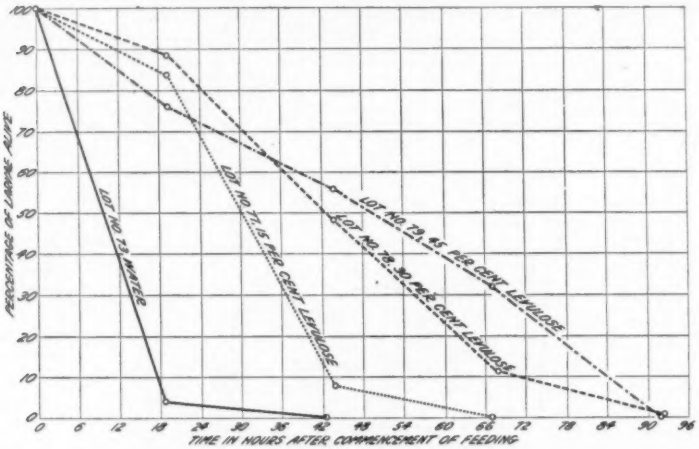

LOT NO 73, WATER
LOT NO 77, 15 PER CENT LEVULOSE
LOT NO 78, 30 PER CENT LEVULOSE
LOT NO 79, 45 PER CENT LEVULOSE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING

FIG. 2.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of levulose in certain concentrations with water, and of one lot supplied with water only

is devoted to a review and a comparison of those lots, showing optimum length of life for each carbohydrate used.

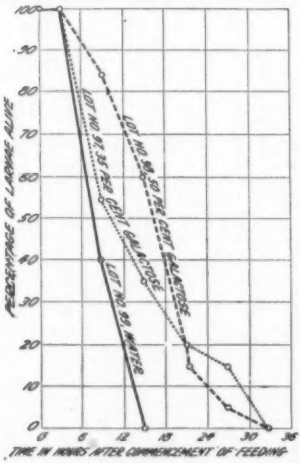


FIG. 3.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of galactose in certain concentrations with water, and of one lot supplied with water only

EXPERIMENTAL RESULTS

MONOSACCHARIDES

Of the monosaccharides, the simplest of the sugars, only the three common representatives were tested—dextrose, levulose, and galactose. All lots fed on dextrose or levulose outlived the larvae fed on water, lot 75, in 30 per cent dextrose, living about four times as long, and lot 79, in 45 per cent levulose, about four and a half times as long, as the lot fed on water (Tables 3, 4, and 5, and figs. 1 and 2). Most of the lots fed on galactose likewise outlived the water check, lot 98, on 50 per cent galactose, living nearly twice as long as lot 99 on water. (Tables 6 and 7 and fig. 3).

It seems evident, then, that the larvae can get nourishment from these simple sugars. The next question is, can the larvae digest other, more complex carbohydrates, changing them to these simple forms? If they sustain themselves on these other carbohydrates it will be considered evidence that they do digest them; if they die in as short a time as they die when starved, it will appear that they do not digest these carbohydrates.

TABLE 3.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on dextrose in specified concentrations with water, and a lot supplied with water only, as a check* [a]

Lot 73 (water)		Lot 74 (15 per cent)		Lot 75 (30 per cent)		Lot 76 (45 per cent)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
18.5	4	19.5	40	19.5	88	19.5	76
42.5	0	43.5	0	44.5	40	44.0	32
				67.5	4	67.0	8
				93.0	0	92.5	0

[a] Data presented graphically in fig. 1.

TABLE 4.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on levulose in specified concentrations with water, and a lot supplied with water only, as a check* [a]

Lot 73 (water)		Lot 77 (15 per cent)		Lot 78 (30 per cent)		Lot 79 (45 per cent)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
18.5	4	20.0	84	20.0	88	20.0	76
42.5	0	44.5	8	44.5	48	44.0	56
		67.0	0	67.5	12	67.0	32
				92.5	0	92.0	0

[a] Data presented graphically in fig. 2.

TABLE 5.—*Average length of life for lots of larvae fed respectively on solutions of dextrose and of levulose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
73	Water	25	19	10.10±0.55
74	15 per cent dextrose	25	18	18.27±1.43
75	30 per cent dextrose	25	16	39.90±2.32
76	45 per cent dextrose	25	18	36.01±2.73
77	15 per cent levulose	25	19	30.57±1.58
78	30 per cent levulose	25	18	39.06±2.12
79	45 per cent levulose	25	19	47.56±3.64

TABLE 6.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on galactose in specified concentrations with water, and a lot supplied with water only, as a check* [a]

Lot 97 (35 per cent)		Lot 98 (50 per cent)		Lot 99 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
3.0	100	3.0	100	3.0	100
9.0	55	9.0	85	9.0	40
15.0	35	15.0	60	15.0	0
21.5	20	21.5	15		
27.0	15	27.0	5		
32.5	0	32.5	0		

[a] Data presented graphically in fig. 3.

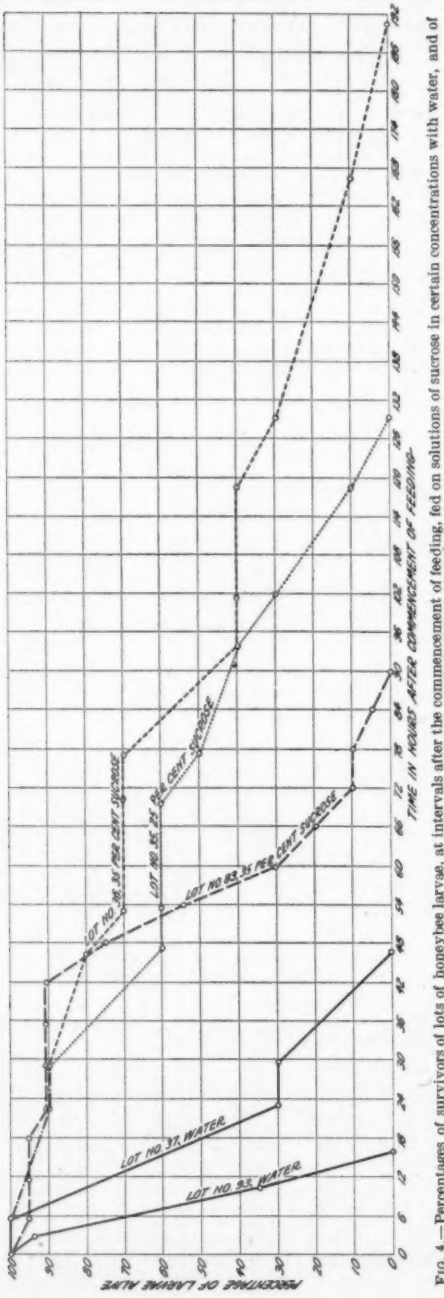

LOT NO. 36, 35 PER CENT SUCROSE
LOT NO. 125, 75 PER CENT SUCROSE
LOT NO. 84, 50 PER CENT SUCROSE
LOT NO. 37, WATER
LOT NO. 93, WATER
PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING.

FIG. 4.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of sucrose in certain concentrations with water, and of one lot supplied with water only.

DISACCHARIDES

The disaccharides commonly used as food by animals are sucrose, maltose, and lactose. Honeybee larvae were fed on all of these, and also on trehalose, to which particular interest is attached in connection with the honeybee. This rare sugar occurs, so far as known, only in a few fungi. The interest here lies in the fact that Phillips found adult honeybees to be able readily to utilize this sugar as food, in spite of the fact that it probably never occurs in their normal diet.

In the experiments here described the larvae lived longer on all concentrations of sucrose than on water, the concentration of 75 per cent being the most favorable; the ratio of the length of life of lot 125 (75 per cent sucrose) to lot 129 (water) is about 15 to 1, that of lot 84 (50 per cent sucrose) to lot 93 (water) 7 to 1, and that of lot 36 (35 per cent sucrose) to lot 37 (water) 4½ to 1. (Tables 8 and 9 and fig. 4.)

On maltose the larvae likewise outlived decidedly the larvae that were starved; e. g., lot 41, on 35 per cent maltose, lived more than five times as long as lot 43, on water, and lot 88, on 35 per cent maltose, lived more than three times as long as its water check, lot 93. (Tables 10 and 11 and fig. 5.)

TABLE 7.—*Average length of life for lots of larvae fed respectively on various concentrations of galactose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
94	5 per cent galactose	20	19	6.8±0.19
95	15 per cent galactose	20	18	8.6±1.86
96	25 per cent galactose	20	19	11.38± .35
97	35 per cent galactose	20	20	13.51±1.26
98	50 per cent galactose	20	17	16.02± .91
99	Water	20	17	8.40± .44

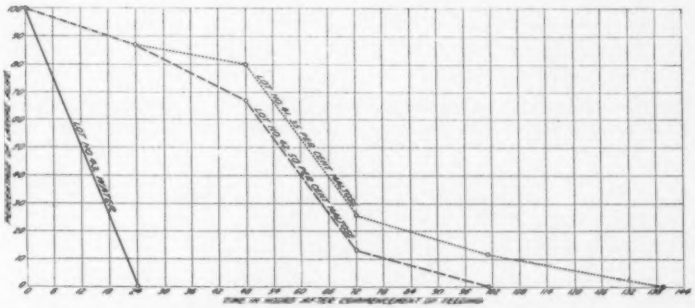


FIG. 5.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of maltose in certain concentrations with water, and of one lot supplied with water only

TABLE 8.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on sucrose in specified concentrations with water, and lots supplied with water only, as checks* [a]

Lot 35 (25 per cent)		Lot 36 (35 per cent)		Lot 37 [b] (water)		Lot 83 (35 per cent)		Lot 93 [c] (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
5.5	100	5.5	100	5.5	100	6.0	95	3.5	95
23.0	90	22.5	90	22.5	30	11.5	95	10.0	35
29.5	90	29.0	90	29.0	30	18.0	95	16.0	0
47.0	60	45.5	80	45.5	0	24.0	90		
53.5	60	53.5	70			30.0	90		
70.5	60	70.0	70			35.5	90		
77.0	50	77.0	70			42.0	90		
94.5	40	94.0	40			48.0	75		
102.0	30	101.5	40			54.0	55		
118.5	10	118.0	40			59.5	30		
129.5	0	129.0	30			66.0	20		
		166.0	10			72.0	10		
		190.0	0			78.0	10		
						84.0	5		
						90.0	0		

[a] Data presented graphically in fig. 4.
[b] Check on lots 35 and 36.
[c] Check on lot 83.

TABLE 9.—*Average length of life for lots of larvae fed respectively on solutions of sucrose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
34	15 per cent sucrose	10	42	45.20± 3.45
35	25 per cent sucrose	10	44	73.12± 8.15
36	35 per cent sucrose	10	41	95.50±11.30
37	Water (check on lots 34, 35, 36)	10	45	21.27± 2.48
80	5 per cent sucrose	20	16	11.80± .62
81	15 per cent sucrose	20	17	17.98± 1.18
82	25 per cent sucrose	20	18	32.90± 2.95
83	35 per cent sucrose	20	18	54.00± 2.82
84	50 per cent sucrose	20	19	59.66± 4.23
93	Water (check on lots 80, 81, 82, 83, 84)	20	18	8.68± .15
125	75 per cent sucrose	20	17	68.85± 7.11
122	100 per cent sucrose	20	14	52.28± 3.47
129	Water (check on lots 125, 122)	20	17	4.61± .23

TABLE 10.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on maltose in specified concentrations with water, and a lot supplied with water only, as a check*[a]

Lot 41 (35 per cent maltose)		Lot 42 (50 per cent maltose)		Lot 43 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
24.0	87	24.0	87	24	0
48.5	80	48.5	67		
72.0	27	72.0	13		
101.0	13	101.0	0		
139.5	0				

[a] Data presented graphically in fig. 5.

TABLE 11.—*Average length of life for lots of larvae fed respectively on solutions of maltose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
38	5 per cent maltose	15	18	15.86±1.16
39	15 per cent maltose	15	24	60.70±5.13
40	25 per cent maltose	15	19	46.95±2.63
41	35 per cent maltose	15	22	63.71±4.78
42	50 per cent maltose	15	21	52.51±3.92
43	Water (check on lots 38, 39, 40, 41, 42)	15	23	12 (between 0 and 24)
85	5 per cent maltose	20	19	11.20± .46
86	15 per cent maltose	20	18	18.40±1.31
87	25 per cent maltose	18	19	23.00±1.47
88	35 per cent maltose	18	17	28.06±2.90
89	50 per cent maltose	20	14	13.87±1.24
93	Water (check on lots 85, 86, 87, 88, 89)	20	18	8.68± .15

The lots fed on lactose were run in two series. In the first series the greatest age was reached by lot 51 (Tables 12 and 13), but since the first examination was not made until 20 hours after the beginning of the experiment, and all the larvae in water were dead, as shown in Figure 6, A, it was impossible to give the length of life of the water check of the first series, lot 52, except as between 0 and 20 hours. For this reason another series of lots was run with examinations at

6-hour intervals. (Fig. 6, B.) In this series all lots showed a longer life on lactose than on water, lot 92, on a saturated solution of lactose,

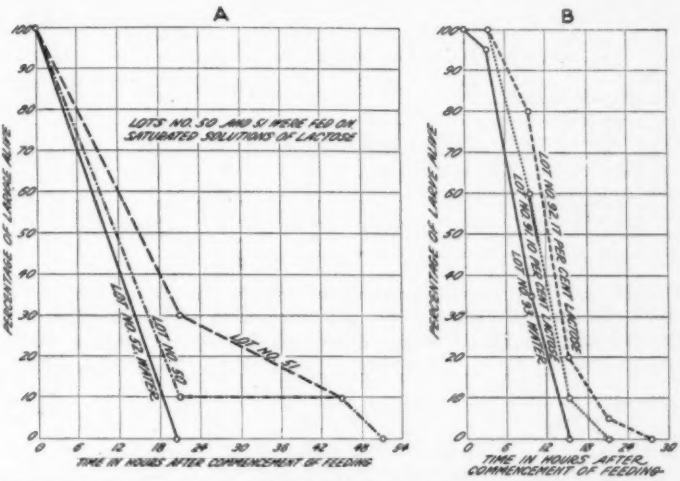

A
B
LOTS NO. 50 AND 51 WERE FED ON SATURATED SOLUTIONS OF LACTOSE
LOT NO. 51
LOT NO. 52
LOT NO. 52, WATER
PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 92, 17 PER CENT LACTOSE
LOT NO. 91, 10 PER CENT LACTOSE
LOT NO. 93, WATER
PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING

FIG. 6.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of lactose in certain concentrations with water, and of lots supplied with water only, as checks. A, two lots, fed on saturated solutions of lactose, and one, supplied with water only; all the larvae of this lot were found to have died in the first interval of 20 hours. B, two lots, fed on lactose in different concentrations, and one, supplied with water only

living 13.18 ± 0.66 hours, as compared with 8.68 ± 0.15 hours for the water check, lot 93, a ratio of about $1\frac{1}{2}$ to 1. This seems to indicate that the larvae are able to digest lactose.

TABLE 12.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on lactose in specified concentrations with water, and lots supplied with water only, as checks* [a]

Lot 50 (saturated solution)		Lot 51 (saturated solution)		Lot 52 [b] (water)		Lot 91 (10 per cent)		Lot 92 (17 per cent)		Lot 93 [c] (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
20.5	10	21.0	30	20.5	0	4.0	100	3.5	100	3.5	95
44.5	10	44.0	10			10.5	60	10.0	80	10.0	35
51.0	0	51.0	0			16.5	10	16.0	20	16.0	0
						22.0	0	21.5	5		
								27.5	0		

[a] Data presented graphically in fig. 6.
[b] Check on lots 50 and 51.
[c] Check on lots 91 and 92.

TABLE 13.—*Average length of life for lots of larvae fed respectively on solutions of lactose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
48	5 per cent lactose	10	17	10 (between 0 and 20).
49	Saturated solution lactose	10	18	12.97±1.12
50	do	10	20	13.95±1.87
51	do	10	22	18.60±3.19
52	Water (check on lots 48, 49, 50, 51)	9	20	10 (between 0 and 20).
90	5 per cent lactose	20	15	11.62± .64
91	10 per cent lactose	20	16	11.57± .59
92	17 per cent lactose	20	15	13.18± .66
93	Water (check on lots 90, 91, 92)	20	18	8.68± .15

The larvae fed on trehalose lived longer on all concentrations tested than they did on water. A comparison of the lengths of life of lot 112 and lot 121 (Tables 14 and 15 and fig. 7) gives a ratio of approximately 3 to 1. It appears, then, that the larvae, like the

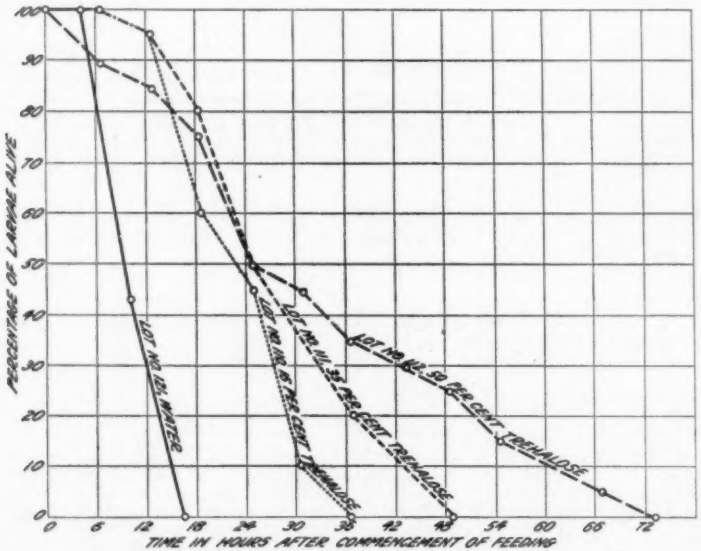

PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 121, WATER
LOT NO. 112, 17 PER CENT TREHALOSE
LOT NO. 111, 10 PER CENT TREHALOSE
LOT NO. 110, 5 PER CENT TREHALOSE

FIG. 7.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of trehalose in certain concentrations with water, and of one lot supplied with water only

adults, can digest this rare sugar with considerable readiness, although it has probably never been a constituent of their diet.

It was found, then, that bee larvae can digest all the disaccharides tested, and that in ability to sustain life these appear to rank in the order sucrose, maltose, trehalose, and lactose.

TABLE 14.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on trehalose in specified concentrations with water, and one lot supplied with water only, as a check* [a]

Lot 110 (16 per cent)		Lot 111 (35 per cent)		Lot 112 (50 per cent)		Lot 121 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
7.0	100	7.0	100	7.0	90	4.5	100
13.5	95	13.5	95	13.5	85	10.5	43
19.5	60	19.5	80	19.5	75	17.0	0
25.5	45	25.5	50	25.5	50		
31.0	10	31.0	35	31.0	45		
37.0	0	37.0	20	37.0	35		
		43.5	10	43.5	30		
		49.5	0	49.5	25		
				55.5	15		
				67.0	5		
				73.5	0		

[a] Data presented graphically in fig. 7.

TABLE 15.—*Average length of life for lots of larvae fed respectively on solutions of trehalose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
110	16 per cent trehalose	20	13	22.95±1.01
111	35 per cent trehalose	20	13	27.75±1.50
112	50 per cent trehalose	20	15	31.17±2.89
121	Water	23	13	10.21± .43

TRISACCHARIDES

The only trisaccharide used in these experiments was melezitose, a sugar occurring in the sap of certain plants and in honeydew. The data indicate clearly that honeybee larvae are able to use melezitose as food, the length of life for lot 104, on 50 per cent melezitose, being about four and one-third times that of lot 109, on water. (Tables 16 and 17 and fig. 8.)

TABLE 16.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on melezitose in specified concentrations with water, and one lot supplied with water only, as a check* [a]

Lot 102 (25 per cent)		Lot 103 (35 per cent)		Lot 104 (50 per cent)		Lot 109 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
6.0	95	6.0	100	6.0	100	6.0	94
12.5	90	12.5	95	12.5	90	12.0	0
18.5	60	18.5	60	18.5	70		
24.5	45	24.5	50	24.5	65		
30.5	20	30.5	35	30.5	55		
36.5	10	36.5	35	36.5	45		
42.5	10	42.5	20	42.5	35		
48.5	0	48.5	15	48.5	30		
		62.5	0	62.5	20		
				67.5	20		
				77.5	0		

[a] Data presented graphically in fig. 8.

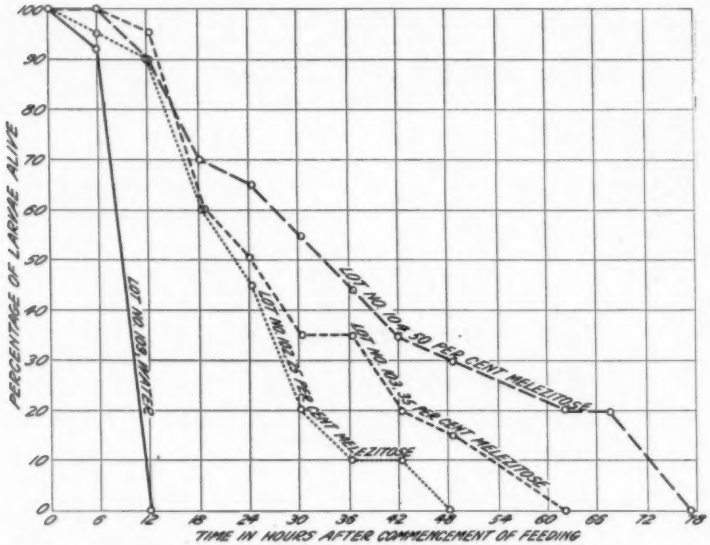

PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 103, WATER
LOT NO. 104, 50 PER CENT MELEZITOSE
LOT NO. 102, 25 PER CENT MELEZITOSE
LOT NO. 101, 10 PER CENT MELEZITOSE
LOT NO. 103, 35 PER CENT MELEZITOSE

FIG. 8.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of melezitose in certain concentrations with water, and of one lot supplied with water only

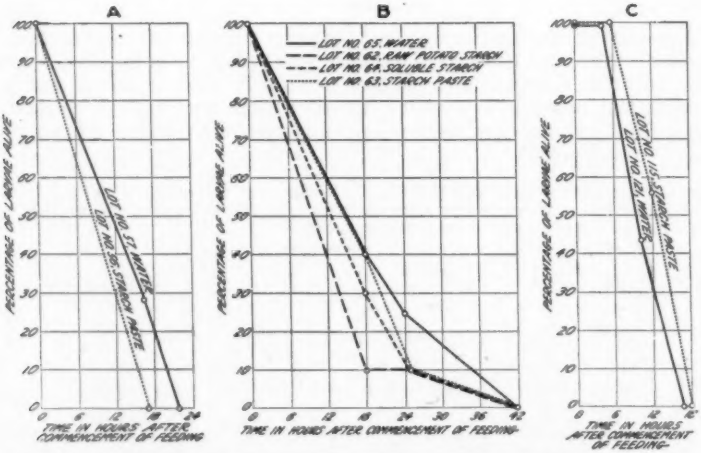

A
B
C
PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 67, WATER
LOT NO. 66, STARCH PASTE
LOT NO. 65, WATER
LOT NO. 62, RAW POTATO STARCH
LOT NO. 64, SOLUBLE STARCH
LOT NO. 63, STARCH PASTE
LOT NO. 155, STARCH PASTE
LOT NO. 120, WATER

FIG. 9.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on combinations of starch and water, and others supplied with water only as checks. A, lot fed on starch paste, and lot supplied with water; B, three lots, fed respectively on raw potato starch, starch paste, and soluble starch, and one lot supplied with water only; C, one lot fed on starch paste, and one supplied with water only

TABLE 17.—*Average length of life for lots of larvae fed respectively on solutions of melezitose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
100	5 per cent melezitose	20	11	9. 25±0. 28
101	15 per cent melezitose	20	11	16. 30±1. 05
102	25 per cent melezitose	20	11	23. 26±2. 56
103	35 per cent melezitose	20	11	28. 58±2. 24
104	50 per cent melezitose	20	11	37. 47±3. 31
109	Water	16	11	8. 62± . 24

POLYSACCHARIDES

The polysaccharides used in these experiments were dextrin, glycogen, and starch. The data obtained for these three carbohydrates show that of the five lots fed on starch only one (lot 115) lived longer than the corresponding water check, and even that one less than two hours longer. (Tables 18 and 20 and figs. 9 and 10.) There was no significant difference between the length of life of the lot fed on glycogen (lot 114) and its water check, lot 121. (Tables 18 and 20 and fig. 10.) The lot fed on dextrin (113), however, lived nearly three times as long as the corresponding lot (121) fed on water alone. (Tables 19 and 20 and fig. 10.)

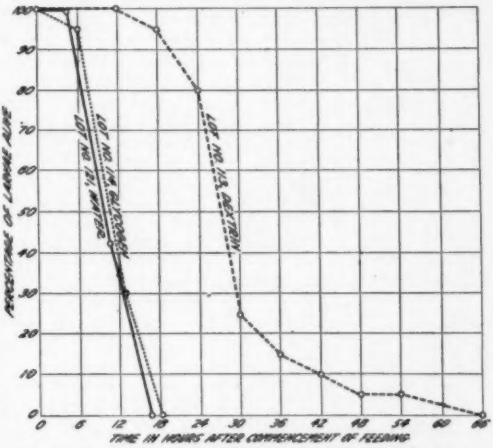

PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 114, GLYCOGEN
LOT NO. 113, DEXTRIN
LOT NO. 121, WATER

FIG. 10.—Percentages of survivors of three lots of honeybee larvae, at intervals after the commencement of feeding; one supplied with water only, as a check, and the others fed on solutions of dextrin and glycogen, respectively

TABLE 18.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on combinations of starch and water, and lots supplied with water only, as checks* [a]

Lot 56 (starch paste)		Lot 57 [b] (water)		Lot 62 (raw starch)		Lot 63 (starch paste)		Lot 64 (soluble starch)		Lot 65 [c] (water)		Lot 115 (starch paste)		Lot 121 [d] (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hrs.	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*	*Hrs.*	*P. ct.*
17	0	16. 5	29	18. 0	10	18. 0	40	18. 0	30	18. 0	40	5. 5	100	4. 5	100
		22. 0	0	24. 5	10	24. 5	10	24. 5	10	24. 5	25	12. 0	55	10. 5	43
				41. 5	0	41. 5	0	41. 5	0	41. 5	0	18. 0	0	17. 0	0

[a] Data presented graphically in fig. 9.
[b] Check on lot 56.
[c] Check on lots 62, 63, and 64.
[d] Check on lot 115.

The results indicate that the larvae are able to utilize dextrin, but neither glycogen nor starch.

It should be added here that an examination of larvae which had been floating on a starch mixture for a few hours showed countless grains of starch in the intestine, and therefore the failure to gain nourishment from starch was not due to a failure to take the starch into the alimentary tract.

TABLE 19.—*Observations to ascertain the relative duration of life of three lots of honeybee larvae, one supplied with water only, as a check, and the others fed on solutions of dextrin and glycogen, respectively* [a]

Lot 113 (30 per cent dextrin)		Lot 114 (50 per cent glycogen)		Lot 121 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
6.0	100	6.0	95	4.5	100
12.5	100	12.5	30	10.5	43
18.5	95	18.5	0	17.0	0
24.5	80				
30.0	25				
36.0	15				
42.5	10				
48.5	5				
54.5	5				
66.5	0				

[a] Data presented graphically in fig. 10.

TABLE 20.—*Average length of life for lots of larvae fed respectively on starch, dextrin, glycogen, and water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	*Hours*
56	Starch paste	10	23	8.5 (between 0 and 17)
57	Water (check on lot 56)	7	23	11.39±1.54
62	Raw starch	20	43	11.40±.85
63	Starch paste	20	42	15.07±1.41
64	Soluble starch	20	46	13.85±1.32
65	Water (check on lots 62, 63, 64)	20	42	16.83±1.91
115	Starch paste	20	15	12.18±.46
114	50 per cent glycogen	20	12	10.81±.50
113	30 per cent dextrin	20	15	29.55±1.24
121	Water (check on lots 115, 114, 113)	23	13	10.21±.43

HONEYS

A study of the ability of honeybee larvae to utilize carbohydrates as food would, of course, be incomplete without an investigation of their relative ability to use honey, which is the main carbohydrate constituent of their normal diet. Bees gather nectar from many sources, and from the different nectars make honeys varying considerably in physical and chemical characteristics. Since, as is well known among beekeepers, these differ in value as food for adult bees, it is probable that they also differ in value as food for the brood.

In order to investigate this question, several lots of larvae were fed on honeys differing as widely as possible in source, color, odor, taste, dextrin content, dextrose-levulose ratio, tannin content, sucrose con-

tent, and the like. These honeys were from sage, white clover, alfalfa, tulip tree, aster, goldenrod, buckwheat, wild buckwheat, mountain laurel, locust, and basswood clover, the latter consisting of three parts of basswood to one of clover. At the same time, for check and comparison, lots were also fed on distilled water and on 75 per cent cane sugar.

The average length of life of the lots fed on the various honeys ranges from 59.82 ± 3.89 hours on basswood clover to 12.39 ± 0.84 hours on locust. (Table 22.) All the honeys tested may be arranged in the following order from greatest to least length of life: Basswood clover, white clover, tulip tree, alfalfa, buckwheat, mountain laurel (poisonous to humans), sage, wild buckwheat, aster, goldenrod, and locust. The average for the 11 honeys tried is 38.93 hours (probable error disregarded), which is essentially the same as for lot 138, fed on 75 per cent cane sugar, and is approximately three and one-half times as long as for lot 139, fed on water alone. (Tables 21 and 22 and fig. 11.)

TABLE 21.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae; one fed on 75 per cent solution of cane sugar, one on locust honey, one on a mixture of 75 per cent of basswood honey and 25 per cent of clover honey, and one supplied with water only as a check* [a]

Lot 140 (75 per cent basswood honey, 25 per cent clover honey)		Lot 142 (locust honey)		Lot 138 (75 per cent cane sugar)		Lot 139 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*	*Hours*	*Per cent*
5.0	100	4.5	92	5.0	96	5.0	100
16.5	96	16.0	16	16.5	72	16.5	0
23.0	96	22.5	12	23.0	72	----	----
28.5	96	28.0	8	28.5	68	----	----
33.5	84	33.0	4	33.5	68	----	----
41.0	68	40.5	0	41.0	44	----	----
47.0	64	----	----	47.0	32	----	----
53.0	48	----	----	53.0	28	----	----
65.0	44	----	----	65.0	20	----	----
71.0	32	----	----	71.0	16	----	----
77.0	28	----	----	77.0	8	----	----
89.0	20	----	----	89.0	4	----	----
95.0	12	----	----	95.0	4	----	----
101.0	8	----	----	101.0	4	----	----
113.0	0	----	----	113.0	0	----	----

[a] Data presented graphically in fig. 11.

These data show no correlation between length of life of the larvae and any known physical or chemical characteristics of the honey. For example, the length of life on the light honeys, white clover, alfalfa, sage, locust, and basswood clover, averaged 41+ hours, which is not significantly different from the average of 39+ hours on the darker honeys, tulip tree, buckwheat, wild buckwheat, and aster; the tulip-tree honey, with high dextrin content,[5] sustained life as long as sage, clover, or alfalfa, with low dextrin content; tulip tree and buckwheat, strong in flavor and odor and giving strong tannin reactions, had about the same effect as the milder

[5] For chemical analysis of honeys, see Browne (*5*).

sage and basswood clover, having very little tannin; sage, with a high dextrose-levulose ratio, was practically equal in life-sustaining value to alfalfa, with low dextrose-levulose ratio.

TABLE 22.—*Average length of life for lots of larvae fed respectively on various honeys, cane sugar, and water*

Lot No.	Food	Number of larvae	Length of life
			Hours
130	Sage honey	25	39.44±3.09
131	White clover honey	25	51.23±3.69
132	Alfalfa honey	25	45.39±4.13
133	Tulip-tree honey	25	50.16±3.25
134	Aster honey	25	24.79±2.09
135	Goldenrod honey	25	18.72±1.69
136	Buckwheat honey	25	44.57±2.40
137	Wild buckwheat honey	25	38.45±2.64
140	Basswood honey, 75 per cent; clover honey, 25 per cent	25	59.82±3.89
141	Mountain laurel honey (poison)	25	43.30±1.74
142	Locust honey	25	12.39± .84
	Average of all honeys		38.93
138	75 per cent cane sugar	25	40.86±3.53
139	Water	25	10.70

[a] The larvae were not weighed, but all lots are comparable as to size and age.

It is a fact, however, that not all honeys were of equal value in sustaining life. This fact may have been due to an unknown characteristic of the honeys, to an uncontrolled factor in the experiment, or to a difference in the larvae themselves.

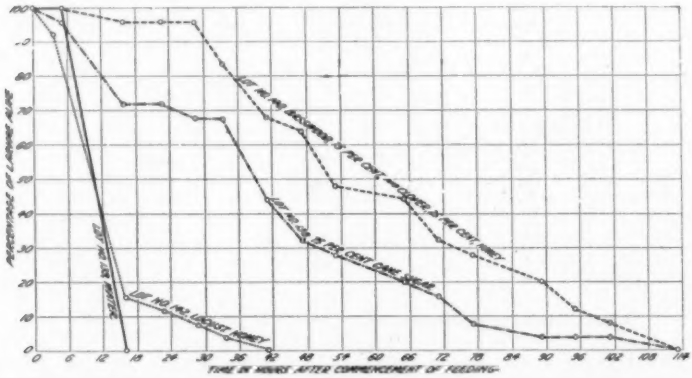

PERCENTAGE OF LARVAE ALIVE
TIME IN HOURS AFTER COMMENCEMENT OF FEEDING
LOT NO. 140, BASSWOOD 75 PER CENT, CLOVER 25 PER CENT, HONEY
LOT NO. 138, 75 PER CENT CANE SUGAR
LOT NO. 142, LOCUST HONEY
LOT NO. 139, WATER

FIG. 11.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding; one fed on a 75 per cent solution of cane sugar, one on locust honey, one on a mixture of 75 per cent of basswood honey and 25 per cent of clover honey, and one supplied with water only.

REVIEW AND COMPARISON

In the preceding pages have been presented in detail the results obtained in experiments on honeybee larvae fed on solutions of the monosaccharides, dextrose, levulose, and galactose; of the disaccharides, sucrose, maltose, lactose, and trehalose; of the trisaccharide, melezitose; and of the polysaccharides, dextrin, starch, and

glycogen; and on various honeys. A comparison of the life-sustaining value of these various carbohydrates can now readily be made by first computing a ratio for each carbohydrate and then comparing the ratios. The ratio in each case is found by dividing the length of life of the optimum lot in each series by the length of life of its particular water check. In case more than one series of lots were run for a single carbohydrate, an average is taken of the ratios of all the series for that carbohydrate. (Table 23.) A ratio of one, or less than one, means, of course, that the length of life on that carbohydrate was equal to or less than the length of life on water, and indicates no food value at all.

TABLE 23.—*Comparison of various carbohydrates as to their value in sustaining the life of honeybee larvae. All carbohydrates tried appear to have food value except starch and glycogen (probable error disregarded)*

Name	Carbohydrate (=C)		Starvation check (water) (=S)		Ratio (C/S)	Average ratio (C/S)
	Lot No.	Length of life	Lot No.	Length of life		
Sucrose	36	95.50	37	21.27	4.5	
Do	84	59.66	93	8.68	6.9	
Do	125	68.85	129	4.61	14.9	
Do	138	40.86	139	10.70	3.8	7.5
Levulose	79	47.56	73	10.10	4.7	4.7
Maltose	41	63.71	43	12.00	5.3	
Do	88	28.06	93	8.68	3.2	4.3
Melezitose	104	37.47	109	8.62	4.3	4.3
Dextrose	75	39.90	73	10.10	4.0	4.0
Honeys	(a)	38.93	139	10.70	3.6	3.6
Trehalose	112	31.17	121	10.21	3.1	3.1
Dextrin	113	29.55	121	10.21	2.9	2.9
Galactose	98	16.02	99	8.40	1.9	1.9
Lactose	51	18.60	52	10.00	1.9	
Do	92	13.18	93	8.68	1.5	1.7
Starch	56	8.50	47	11.39	.7	
Do	63	15.07	65	16.83	.9	
Do	115	12.18	121	10.21	1.2	.9
Glycogen	114	10.81	121	10.21	1.1	1.1

[a] Average.

These data show (Table 23) that sucrose, with a ratio of 7.5, had the greatest life-sustaining value, and that following this came, in order, levulose (4.7), maltose (4.3), melezitose (4.3), dextrose (4.0), honeys (3.6), trehalose (3.1), dextrin (2.9), galactose (1.9), lactose (1.7), glycogen (1.1), and starch (0.9). Glycogen and starch, therefore, had no more food value than distilled water.

ENZYMES

Practically all work done on digestion in insects has been a study of the various enzymes produced in the insect, either in the body as a whole or in some particular part of it. If the results obtained by feeding are to be compared with results obtained by investigating the enzymes, it follows that both must be reduced to common terms.

Our knowledge of digestion in general indicates that an organism which is able to get nourishment from a carbohydrate other than a monosaccharide must first change this carbohydrate to a monosaccharide. Likewise, our knowledge of enzymes indicates that a specific enzyme is necessary for the hydrolysis of each complex carbohydrate

to a simpler form. It therefore seems clear that if an insect is able to obtain nourishment from a complex carbohydrate it must produce the specific enzyme necessary to hydrolyze this carbohydrate, and it must secrete this enzyme at a place where it may be in contact with the food.

To state the results of these experiments in terms of enzymes, it seems clear that the honeybee larva has available for use in digestion, the following enzymes: Invertase, maltase, melezitase, trehalase, dextrinase, and lactase, but does not have available any diastase.

The fact that there is no diastase available for use in digestion was quite unexpected, in view of the apparent widespread distribution of diastase among insects and its presence even in the adult honeybee, as reported by Erlenmeyer, Axenfeld, Petersen, and Pavlovsky and Zarin. (Table 1.) But is it not possible for diastase to be produced in an insect and still not be secreted into the intestine?

In order to obtain direct evidence concerning this question a number of larvae were punctured and their coelomic fluid (blood) collected. A few cubic centimeters of this fluid were added to a thin starch paste and incubated overnight at a temperature of 37.5 °C. The next morning the starch had disappeared and the solution gave a distinct reaction to Fehling's test for sugar, thus showing clearly that diastase is present in the coelomic fluid. If, now, it be recalled that, as before mentioned, the larvae take starch into their alimentary tract, but receive no nourishment from it, it seems evident that although diastase is present in the larva as a whole, it is not present in the lumen of its digestive tract. This conclusion is essentially the same as that of Phillips (*17*) for the adult honeybee.

At the same time, tests were made to ascertain whether or not invertase, maltase, and lactase were present in the coelomic fluid. Coelomic fluid was added to solutions of pure sucrose, maltose, and lactose, and the hydrogen-ion concentration was adjusted to P_H 6.8. Then the specific rotation of the solution was ascertained by means of the polariscope, after which the solution was covered with toluol and allowed to stand for 24 hours. The polariscope reading was then again taken but in no case was any significant change noted. Such negative results give an indication, although by no means a proof, that neither invertase, maltase, nor lactase is present in the coelomic fluid of the larva, although from feeding experiments these enzymes are shown to be available for use in digestion.

SUMMARY AND CONCLUSIONS

Honeybee larvae may be removed from the comb and kept alive under artificial conditions such as are described under the head, "Material and method."

They are able to utilize as food the following carbohydrates, named in the order of their apparent food value: Sucrose, levulose, maltose, melezitose, dextrose, trehalose, dextrin, galactose, and lactose (the last very slightly). This fact indicates that the larva has available the digestive enzymes invertase, maltase, melezitase, trehalase, dextrinase, and lactase. Honeybee larvae apparently differ from adult honeybees, then, in their ability to use dextrin and galactose (according to Phillips) and lactose (according to both Phillips and Pavlovsky and Zarin). Lactase, has, however, been

found in the larvae of *Euproctis chrysorrhoea*, *Bombyx mori*, *Hyponomeuta* sp., and *Tenebrio molitor*. (Table 1.)

Two carbohydrates which honeybee larvae are not able to use as food are starch and glycogen. The enzyme diastase is therefore not present in the lumen of the gut. But the enzyme diastase is present in the coelomic fluid of the larvae, as shown by the ability of the coelomic fluid to digest starch. In this respect, according to Phillips, the larvae are like the adult honeybees; according to all other investigators they are unlike the adults. They are also apparently unlike almost every other insect which has been investigated.

Larvae are like adults in their ability to metabolize two rare sugars, melezitose and trehalose. Their ability to use trehalose is especially remarkable in view of the fact that this sugar probably never occurs in their normal diet.

Under the conditions of these experiments honeys show a life-sustaining value slightly less than sucrose, and approximately equal to levulose, maltose, melezitose, and dextrose. The various honeys used ranked in the following order from greatest to least life-sustaining value: Basswood clover, white clover, tulip tree, alfalfa, buckwheat, mountain laurel, sage, wild buckwheat, aster, goldenrod, and locust; the reason for this order of values is not clear, since it is not to be explained on the basis of any known physical or chemical characteristic of the honeys.

LITERATURE CITED

(1) Axenfeld, D.
1903. invertin im honig und im insektendarm. Zentbl. Physiol. 17: 268–269.

(2) Basch, S.
1858. untersuchungen über das chylopoetische und uropoetische system der blatta orientalis. Sitzber. K. Akad. Wiss. Wien, Math. Naturw. Kl. 33: 234–260, illus.

(3) Biedermann, W.
1898. beiträge zur vergleichenden physiologie der verdauung. I. die verdauung der larve von tenebrio molitor. Pflüger's Arch. Physiol. 72: 105–162, illus.

(4) ———
1910. die aufnahme, verarbeitung und assimilation der nahrung. neunter teil. die ernährung der insekten (hexapoda). *In* Winterstein, H., Handbuch der vergleichenden Physiologie, Bd. 2, p. 726–902, illus. Jena.

(5) Browne, C. A.
1908. chemical analysis and composition of american honeys. U. S. Dept. Agr., Bur. Chem. Bul. 110, 93 p., illus.

(6) Chapman, R. N.
1924. nutritional studies on the confused flour beetle, tribolium confusum duval. Jour. Gen. Physiol. 6: 565–585, illus.

(7) Cleveland, L. R.
1924. the physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to reticulitermes flavipes kollar. Biol. Bul. Mar. Biol. Lab. Woods Hole 46: 178–227.

(8) Erlenmeyer, [E.]
1874. ueber die fermente in den bienen, im bienenbrot und im pollen und über einige bestandtheile des honigs. Sitzber. K. Akad. Wiss. München, Math. Phys. Kl. 4: 204–207.

(9) Frenzel, J.
1882. ueber bau und thätigkeit des verdauungskanals der larve des tenebrio molitor mit berücksichtigung anderer arthropoden. Berlin. Ent. Ztschr. 26: [267]–316, illus.

(10) GRUNER, M.
1900. BEITRÄGE ZUR FRAGE DES AFTERSECRETES DER SCHAUMCICADEN. Zool. Anz. 23: 431–436.
(11) JOUSSET [DE BELLESME].
1876. RECHERCHES SUR LES FONCTIONS DES GLANDES DE L'APPAREIL DIGESTIF DES INSECTES. Compt. Rend. Acad. Sci. [Paris] 82: 97–99.
(12) KELLNER, O.
1884. CHEMISCHE UNTERSUCHUNGEN ÜBER DIE ENTWICKLUNG UND ERNÄHRUNG DES SEIDENSPINNERS (BOMBYX MORI). Landw. Vers. Sta. 30: 59–86.
(13) KOBERT, R.
1903. UEBER EINIGE ENZYME WIRBELLOSER THIERE. Pflüger's Arch. Physiol. 99: 116–186.
(14) NELSON, J. A., and STURTEVANT, A. P.
1924. GROWTH AND FEEDING OF HONEYBEE LARVAE. PART I. THE RATE OF GROWTH OF THE HONEYBEE LARVA. U. S. Dept. Agr. Bul. 1222: 1–24, illus.
(15) PAVLOVSKY, E. N., and ZARIN, E. J.
1922. ON THE STRUCTURE OF THE ALIMENTARY CANAL AND ITS FERMENTS IN THE BEE (APIS MELLIFERA L.). Quart. Jour. Micros. Sci. [London] (n. s. No. 263) 66: [509]–556, illus.
(16) PETERSEN, H.
1912. BEITRÄGE ZUR VERGLEICHENDEN PHYSIOLOGIE DER VERDAUUNG. V. DIE VERDAUUNG DER HONIGBIENE. Pflüger's Arch. Physiol. 145: 121–151, illus.
(17) PHILLIPS, E. F.
1924. THE DIGESTION OF THE HONEYBEE. Gleanings Bee Cult. 52: 76–79.
(18) PLATEAU, F.
1875. RECHERCHES SUR LES PHÉNOMÈNES DE LA DIGESTION CHEZ LES INSECTES. Acad. Roy. Belg., Mém. Cl. Sci., t. 41, pt. 1, 124 p., illus.
(19) ———
1876. NOTE SUR LES PHÉNOMÈNES DE LA DIGESTION CHEZ LA BLATTE AMÉRICAINE (PERIPLANETA AMERICANA, L.). Acad. Roy. Belg., Bul. Cl. Sci. (2) 41: 1206–1233.
(20) SEILLIÈRE, G.
1905. SUR UNE DIASTASE HYDROLYSANT LA XYLANE DANS LE TUBE DIGESTIF DE CERTAINES LARVES DE COLÉOPTÈRES. Compt. Rend. Soc. Biol. [Paris] 57 (1): 940–941.
(21) SIEBER, N., and METALNIKOW, S.
1904. UEBER ERNÄHRUNG UND VERDAUUNG DER BIENENMOTTE (GALLERIA MELLONELLA). Pflüger's Arch. Physiol. 102: 269–286.
(22) SITOWSKI, L.
1905. BIOLOGISCHE BEOBACHTUNGEN ÜBER MOTTEN. Bul. Internatl. Acad. Sci. Cracovie, Cl. Sci. Math. et Nat. 1905: 534–548, illus.
(23) STRAUS, J.
1908. ÜBER DAS VORKOMMEN EINIGER KOHLEHYDRATFERMENTE BEI LEPIDOPTEREN UND DIPTEREN IN VERSCHIEDENEN ENTWICKLUNGSSTADIEN. Ztschr. Biol. (n. F. 34) 52: [95]–106.
(24) SWINGLE, H. S.
1925. DIGESTIVE ENZYMES OF AN INSECT. Ohio Jour. Sci. 25: 209–218.
(25) WEINLAND, E.
1905. ÜBER DIE AUSSCHEIDUNG VON AMMONIAK DURCH DIE LARVEN VON CALLIPHORA, UND ÜBER EINE BEZIEHUNG DIESER TATSACHE ZU DEM ENTWICKLUNGSSTADIUM DIESER TIERE. Ztschr. Biol. (n. F. 29) 47: 232–250.
(26) ———
1907. WEITERE BEOBACHTUNGEN AN CALLIPHORA. IV. ÜBER CHEMISCHE MOMENTE BEI DER METAMORPHOSE (UND ENTWICKLUNG). Ztschr. Biol. (n. F. 31) 49: 486–493.

A ROOT ROT OF LUCRETIA DEWBERRY CAUSED BY A VARIETY OF COLLYBIA DRYOPHILA FR.[1]

By R. F. POOLE

Associate Plant Pathologist, North Carolina Agricultural Experiment Station

INTRODUCTION

The culture of the Lucretia dewberry *Rubus procumbens* Muhl. var. *roribaccus* Bailey was begun as a commercial enterprise about 25 years ago in the sand hills, where it is planted from Sanford to Hamlet in North Carolina. Although the industry has increased rapidly, the plant in many fields has lately become badly diseased and at times unprofitable (*11*).[2] The most recent trouble is a root rot that is certain to become a much greater economic factor in growing berries because it severely reduces the productiveness of the plants, and by causing the death of some plants leaves a ragged irregular stand, which has not been corrected by cultural practices. It has been necessary to remove plants affected by root rot and the loss has become of such economic importance that studies were begun for the purpose of determining the cause and its control. These studies have revealed an undescribed disease of the dewberry and a causal fungus heretofore unknown as a plant parasite.

The symptoms of the disease, the life cycle of the causal organism, its dissemination, and parasitism are described.

HISTORY AND DISTRIBUTION

The root-rot disease was called to the attention of G. W. Fant, extension plant pathologist, during the growing season of 1922. The first reports were from Cameron, but investigations in 1926 revealed the fact that the disease was widespread throughout the sand hills, where it is apparently becoming worse. It is causing greater damage in some areas than in others; in some fields from 15 to 90 per cent of the plants are diseased. In the vicinity of Cameron the infection since 1922 has been so severe in some fields that all plants have been plowed out because of a diseased, unprofitable condition and the infected soil has since that time been used for other crops such as cotton and tobacco, which do not seem to be affected.

The origin of the disease is not known but may easily be the present dewberry fields, since there is no report of this disease occurring elsewhere. Coker and his associates (*2*) have collected sporophores from various habitats in many localities of the State that are very similar to the fruiting bodies of the causal organism. Furthermore, the same investigators have found sporophores very similar to the causal organism in oak and pine woods, which make up the natural vegetation of a greater portion of the sand hills. However, there are no acceptable data to show that the fungus has been a parasite on the previous vegetation of lands now used for planting the dewberry.

[1] Received for publication Apr. 12, 1927; issued October, 1927. Published with the approval of the Director of the North Carolina Experiment Station as paper No. 18 of the Journal series.

[2] Reference is made by number (italic) to "Literature cited," p. 464.

While the disease appears to be confined to the sand hills in North Carolina, other varieties of the causal fungus are widely distributed geographically according to Buller (*1, p. 465, 470*), who says: "*Collybia dryophila* is a well-known agaric commonly found in woods, both in Europe and North America. . . . In English woods, especially those of Sutton Park, near Birmingham, in the course of 20 years I must have seen some thousands of *C. dryophila*." McIlvaine (*6*) reported it from West Virginia, North Carolina, New Jersey, and Pennsylvania. In the latter State he reported a very pretty form, which was found on pine needles at Eagles Mere. Kauffman (*4*) found it very common throughout the State of Michigan, where the typical forms were in hardwood forests. There are slight differences

FIG. 1.—A, Field of Lucretia dewberries badly infected with root-rot disease (July 15): *a*, wilted canes on diseased plant; *b*, empty hill, plant set in which was killed by the disease. B, field of Lucretia dewberries August 15 after old canes were removed, showing: *a*, weak plants due to diseased roots; *b*, where plant was killed by the disease. C, Field of Lucretia dewberries July 15, showing healthy growth of canes on uninfected roots. D, Diseased canes, showing: *a*, girdling of cane; *b*, death of cane due to complete infection of all parts below; *c*, cane spur left after pruning, through which infection is common; *d*, small section of live tissue supporting weak cane growth above; *e*, small and bushy leaves

noted in descriptions of the *C. dryophila* by these authors, which are obvious, since McIlvaine (*6, p. 121*) says: "*C. dryophila* is so common and variable that descriptions would fail to cover it in its eccentricities," and Kauffman (*4, p. 755*) speaks of the *C. dryophila* "group" as "a whole series of variations," and he further believes that its varieties appear under different influences of habitat. This remarkable history of the fungus indicates that a parasitic variety could easly be confined to isolated areas without being distributed as widely as other varieties of *C. dryophila*.

The fungus is carried on roots of new plants, this being determined by isolating the fungus from plants developed in infected soils and by inoculating new plants. The white to tan colored rhizomorphs are sometimes prominent on the plants grown on infected soils. The

fungus is easily disseminated from one plant to another in the same field on pruning shears, which are used in July to cut out all canes to or just beneath the soil. It is quite obvious that the inoculum is disseminated in other ways in which the soil is disturbed.

SYMPTOMS OF DISEASE

To the casual observer the most obvious symptom is the death of plants following severe infection (fig. 1, A and B), and the ragged, irregular growth of canes in comparison to healthy plants (fig. 1, C). This is not the first nor the most significant symptom, however, since the plant may be severely stunted and may produce a very inadequate growth of canes and yield only a small quantity of berries for one or many seasons before death occurs. (Fig. 2.) The causal organism is confined to the parts of the plant at or below the soil level, where it produces an abundance of mycelium, which girdles the canes (fig. 1, D) and finally penetrates the tissues. The girdling is most severe on spring w od (fig. 3, A), but occurs also on the summer and fall wood (fig. 3, B). At this time the roots are also severely attacked and girdled by the fungus (fig. 4, A) and the separated parts are destroyed by an abundant growth of the fungus (fig. 4, B). When badly infected, the normal growth of the plant is arrested and wilting and sudden death are the results. (Fig. 3, B.) This condition may occur when the canes are young or, as is often the case, just as the berries are beginning to ripen. In the latter event the berries are inferior in quality or totally worthless. (Fig. 5.) The plants have a natural inclination to resist the disease by the production of new canes from the roots that extend below the infected parts. When the original stump is killed, a new one develops, and when this is killed, still another is produced. The process of death and production of new canes is often repeated a number of times, so that single plants having several dead stumps are often found. (Fig. 6, A.) Severely diseased stumps are unable to properly nourish the canes; consequently the latter remain short and bushy and may readily be distinguished by contrast with the more vigorous, more productive canes produced on healthy plants. (Fig. 2.)

FIG. 2.—Healthy plants on left, right, and in background; diseased plants with short canegrowth in the foreground

The foliage on the diseased canes is somewhat crowded, small, and has a purplish hue (fig. 1, D) in contrast to the green on the healthy plants.

When the stumps, roots, and canes of diseased plants are examined, rhizomorphs, mycelium, and wefts of white, fungous hyphae of the causal fungus are easily found throughout the year. (Fig. 6, B.) When the fungus has lived on the same rootstock for several years, the weft of fungos hyphae in the bark and cambium become very pronounced. (Fig. 6, C.) The fungus parts are white or some shade of tan or brown and the wood rot is noticeable also after the fungus enters the old spurs and penetrates downward into the live

FIG. 3.—A, Plants inoculated with a pure culture of the causal organism in August, 1926, showing: *a*, mycelium and rhizomorphs on 1926 cane in April; *b*, fungus on 1927 cane in April. Formed in March, many of these canes were killed before May 1, 1927. B, Diseased plants, showing: *a*, sudden wilting due to complete infection of stem and parts of root system; *b*, uninfected stem and root system; *c*, infection of spur at surface of soil, where wilting and death of several canes in the center of this plant is due to the killing of the tissues between canes and stem by the disease

wood (fig. 6, D), causing its destruction (fig. 7, A). At this stage the strands of hyaline mycelium are present in the xylem, sometimes almost completely filling the cell. (Fig. 7, B.)

THE CAUSAL ORGANISM AND ITS LIFE HISTORY

The disease is caused by a parasitic variety or strain of *Collybia dryophila* Fr. This fungus lives over in and on the tissues of the infected roots and canes in the soil, and there it remains active throughout the winter months, as well as during the growing season. The fact that the fungus was isolated from infected parts early in March, and at other periods throughout the year, is definite proof that the fungus parts are not destroyed by high and low temperatures prevailing in this area during the summer and winter months. The hibernating of the mycelium in the tissues is certain to maintain the life cycle of the fungus for a few if not for many years without the

aid of the spores, since no sporophores were found on dewberry plants in the field during 1926, which was probably due to a severe summer drought. In spite of this fact the fungus was isolated from diseased plants collected in the field in the early part of March of both the 1926 and 1927 seasons.

The fungus is readily obtained in pure culture by plating on prune agar in the usual manner strands of mycelium from rhizomorphs taken from live roots, to escape saprophytes on dead tissues. The prune medium is composed of 10 grams of agar, 125 grams of large prunes, and 1 liter of water. On this medium the mycelium is nearly pure white for 30 days, and sometimes longer, finally becoming creamish and shades of tan. Although the fungus is isolated with some difficulty when neutral media of the ordinary strength of plant coctions and synthetic combinations are used, it grows on these media when large amounts of inoculum are employed or when made slightly acid. The mycelium grows well on rice, potato, sweet potato, apple, corn meal, and Cook's synthetic medium. The mycelium development in barnyard manure is especially well suited for inoculation tests because of the ease with which the inoculum is removed from culture flasks and placed around small roots and stems. While the growth of the mycelium is very abundant on a large number of media, the sporophores have been obtained on parts of the dewberry plant only. This medium was prepared by cutting sections of roots and canes so that the parts could be easily inserted into 2-liter flasks. The medium was moistened, sterilized in the usual manner, and held for 15 days to assure complete sterilization, after which a pure culture of the fungus was added. The culture was incubated at 25° C. in candle light. In 48 hours the growth of the mycelium was evident, and in another 48 hours the rhizomorphs were forming in all parts of the flask. Sporophores began to develop in 30 days, but on the

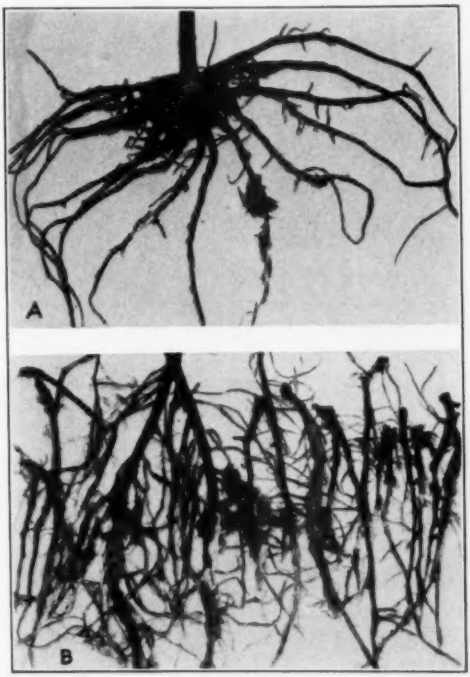


FIG. 4.—A, Results of inoculating new plants. Lesions shown on diseased roots and white mycelium developed abundantly 30 days after inoculation. B, Diseased roots taken from inoculated plants, showing lesions, destruction of roots, and the prolific growth of mycelium

dewberry parts alone they developed slowly and were not always well developed. When prune medium was added to the dewberry parts, it increased the growth of mycelium, rhizomorphs, and sporophores. The color of the mycelium and rhizomorph on plants in the field and in cultures in the laboratory is similar in every way. The development of the mycelium and rhizomorphs has much to do with the production of sporophores, since the largest number of sporophores were obtained when the rhizomorphs were most abundant. Sterile water was added to all flasks at frequent intervals in order to extend the growth of the mycelium and rhizomorphs and thus to determine the duration of sporophore production on the medium used. In these

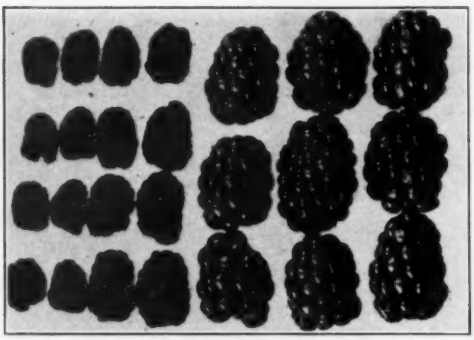


FIG. 5.—Normally ripened berries from uninfected canes compared with those obtained from wilted canes on the same plant as that shown in fig. 3, B, *b*

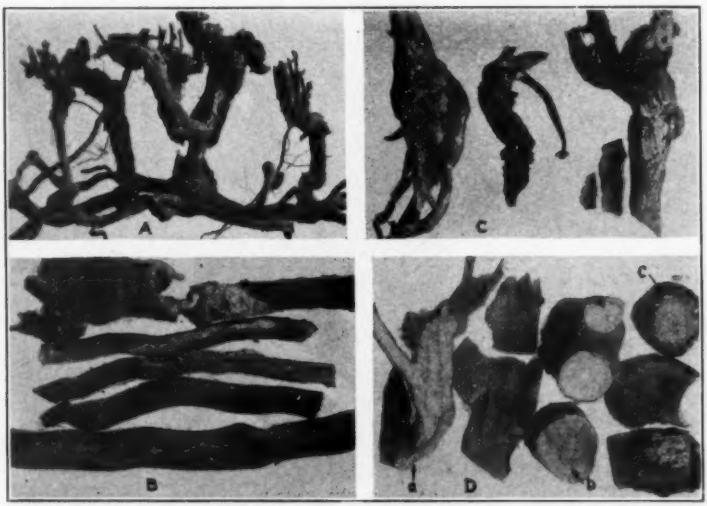


FIG. 6.—A, Underground parts of dewberry plant 10 or more years old, showing dead stumps in center and live but diseased stumps on each end. The central stumps without cane spurs have been dead for several seasons. B, Rhizomorphs and mycelium on roots and stump. C, The results of pure culture inoculation in flasks, showing characteristic weft of white fungous hyphae in bark and cambium of severely infected stems. Note small undeveloped sporophore in center. D, Diseased stems; showing: *a*, slight uninfected area supporting canes above; *b*, slightly discolored diseased heartwood; *c*, transverse section of stem and abundance of white fungous hyphae in both bark and cambium

studies new rhizomorphs and sporophores were produced for six months, after which neither rhizomorphs nor sporophores developed

in these cultures. This was obvious, since an examination of the dewberry parts used in the cultures showed that all tissues of the roots and canes were infected throughout with mycelium which had used the sustenance available to the fungus. Tests for starch and for reducing sugars gave negative results.

In cultures the mycelium is some shade of white or light tan. There are few to many strands of mycelium in a rhizomorph, and these are characterized by long, straight walls and clamp connections. In the cortex, phloem, ray tissue, and cambium the fungus produces wefts or fans of white fungous hyphae, while in the xylem and pith cells it forms single hyaline strands. The rhizomorph is a shade of white, tan, or brown, never black, being alike in pure culture and on infected parts in the soil. It is much branched and extends from one part to another in long straight strands. The largest rhizomorph obtained in these studies measured a millimeter in diameter.

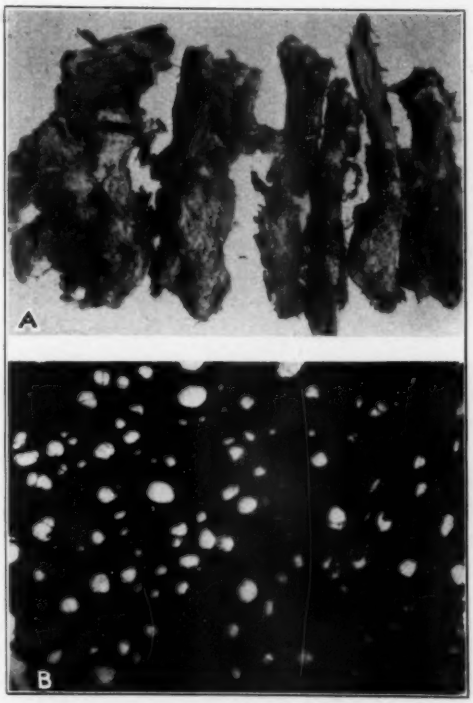


FIG. 7.—A, The result of pure culture inoculation, showing breaking up of roots and stems, which is easily accomplished at the end of six months' culture, due to destructiveness of the fungus. B, Hyaline mycelium in xylem show abundance of strands in some cells

By using the dewberry medium in 10 flasks, an abundance of sporophores were produced. The total production amounted to 138 mature and many immature sporophores. Twenty-eight plants were obtained in a single flask. This heavy production of sporophores has been of much assistance in the determination of the fungus. Specimens were submitted to C. H. Kauffman, of the University of Michigan, G. W. Martin, of the University of Iowa, and W. C. Coker, of the University of North Carolina, all of whom determined the fungus as a variety of *Collybia dryophila* Fr. The sporophore is very similar, and is probably the same as specimens collected by Coker and his associates in various habitats of North Carolina. Therefore, it seems advisable to add a concise but short description of the parasitic variety.

The sporophore as produced in pure culture is solitary, gregarious, and subcaespitose. (Fig. 8, A and B.) The pileus is gibbous,

smooth, dull, subhygrophanous, white, tan, and brown, 2 to 8 centimeters broad. The lamellae are adnexed, crowded, white. The stipe is whitish changing to same color as pileus, stuffed, striate, 3 to 15 centimeters long. Spores are faint buff, pip-shaped, smooth, 4μ to 8μ.

INOCULATION RESULTS

The growth of a weft of fungous hyphae in bark, phloem, ray tissue, and cambium, and the mycelium in the xylem and pith cells of live wood, and persisting there throughout the year, the mass of mycelium forming on and causing girdling of live epidermal cells of that part of the cane in the soil, and the conspicuous rhizomorph on live roots and stems, are prominent suspicious parasitic relations of the fungus to the plant. In order to prove its parasitism, healthy plants were inoculated with a pure culture of the parasitic variety of *Collybia dryophila*. The culture for this inoculum was 60 days old, and was grown on barnyard manure. The inoculum was placed on the roots and stems and the plants set in sand in pots in a greenhouse, where temperature and moisture conditions suitable for excellent growth of the plant and the fungus were maintained, field experiments having shown that the conditions which favored the growth of the plant also favored the development of the fungus as a parasite. Three separate inoculation tests were conducted, each with 10 or more replications. Untreated plants were maintained under similar conditions. In all three tests the results were the same, in that 100 per cent infection was obtained on inoculated plants. The fungus produced lesions on the young roots in seven days (fig. 4, A and B), and in six months the symptoms were identical with those produced in the field (fig. 3, A). However, these studies indicate that several years are required for the complete infection and destruction by the fungus of plants grown under field conditions, but that it may kill canes in a single season. In these tests 9 per cent of the

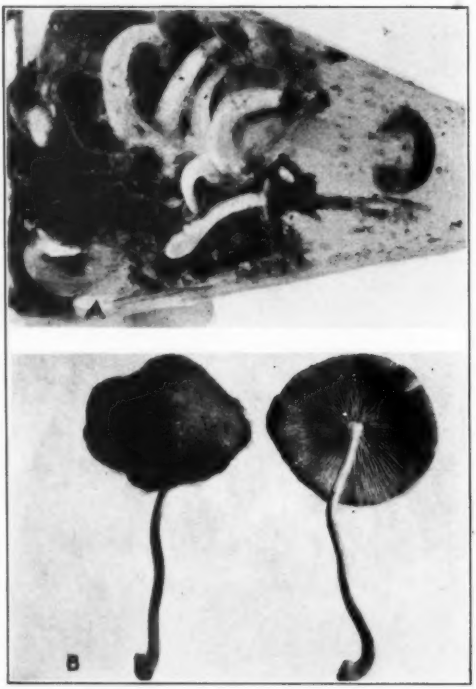


FIG. 8.—A. Pure culture development of sporophores on dewberry medium in 2-liter flask. B. Characteristic sporophore of the parasitic variety of *Collybia dryophila*

plants were killed outright in 30 days because of badly infected roots. On the other hand 68 per cent of the infected plants have remained severely stunted while the fungus continued to attack and live on the roots nine months after the inoculum was added. (Fig. 3, A.) This behavior of the fungus under experimental conditions is similar to its behavior in nature, since the same rootstock is sometimes used for 15 years and longer, and may harbor the fungus causing continuous annual stunting of the plant without causing death. In fact the percentage of plants killed in severely infected areas is generally very small, because the deeper roots, which are not so readily destroyed, continue to produce new canes. The fungus entered the new roots without the aid of mechanical injury, but penetrated broken ends and other injured parts with equal rapidity.

In the field, infection occurs largely through the cane stubs which remain after pruning. This is easily traced on recently infected plants. The fungus enters the dead tissue, later passing into the live tissue, and as a consequence canes are killed at various periods during the growing season. (Fig. 3, B.) In this experiment the fungus girdled the small roots, which died. Later the fungus formed a felt of white hyphae on the infected parts from which the rhizomorphs extended outward into the soil. When the small infected plants were removed from the pots, mycelium and rhizomorphs were found to break off and remain in the pots. The middle lamella of cells of small roots was destroyed, and the tissues as a whole became severely infected. On the stems, the mycelium first worked into the bark, cambium, and through the rays to xylem and pith cells. (Fig. 9, A, B, C, D.) These were most conspicuously occupied by the fungus when the parts were inoculated in flasks. The fungus was isolated in April, 1927, from plants inoculated in August, 1926. and had made continuous growth on the roots and in the tissues throughout the nine months. Although apparently no gum had formed on infected tissues the adhering of soil to the plant seemed to be due partly to some excrement and partly to the matting of mycelium around the small particles. (Fig. 3, B.) Another test was conducted with live roots, which were treated for one minute in a one one-thousandth solution of murcuric chloride, washed in sterile water and added to a pure culture of the fungus in the prune medium. The same procedure was carried out with other sections, which were left uninoculated. The disease and the symptoms were readily obtained on the live roots which were killed, while the uninoculated roots remained healthy and produced new sprouts. In another test similar to this, in which partly live and dead wood was first sterilized and then inoculated, the weft of hyphae became equally if not more pronounced than it is in the field. (Fig. 6, C, and fig. 9, A, B, C, D.) In all of the inoculation studies the results were more or less prominent and easily diagnosed, since the familiar mycelium, hyphae, and rhizomorphs made identification very much more certain than is possible in the study of disease symptoms produced by less conspicucous types of bacteria and fungi. Because it was possible to maintain a continuous water supply and temperature conditions satisfactory for the best growth of the fungus the definite breaking down of the tissues such as was obtained in these pure-culture studies in the laboratory, in which the wood became soft (fig. 7, A), is a

greater demonstration of the power of the fungus to destroy wood than is found on most of the severely infected plants in the field. After six months the diseased wood in the flasks is now easily torn and separated because of the action of the disease on the tissues. The untreated plants in all tests remained healthy throughout and have produced new and vigorous canes, which have been easily distinguished from the shorter and weaker growth of the diseased plants.

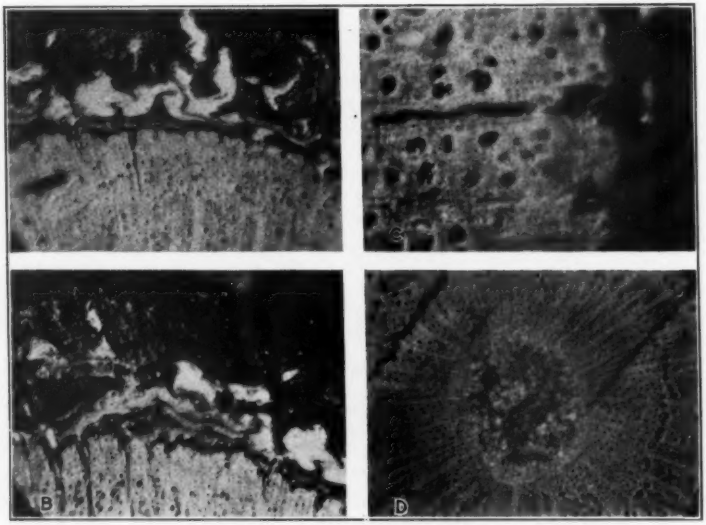


FIG. 9.—Inoculated stem of 4-year old dewberry plant, showing transverse sections made nine months after stems were inoculated with pure culture in laboratory. A, Wefts of fungous hyphae in bark and cambium. B, Fungous hyphae in the cambium, and ray cells of the growth wood. C, Enlarged ray containing strands of mycelium. D, Pith and xylem cells with hyaline mycelium.

DISCUSSION

This is the first report of a parasitic variety of *Collybia dryophila* Fr., a fungus which is well known and has been studied by many mycologists (*1*, *2*, *4*, *6*, *7*, *8*). While McIlvaine and MacAdam (*6*) refer to the fungus as "oak-loving," they do not indicate whether or not it is a parasite on the oak.

The root-rot disease of the dewberry seems identical with the root rots of blackberry and raspberry caused by *Armillaria mellea* (Vahl) Quel. (*5*, *9*) and to the root rot of fruit trees caused by *Clitocybe parasitica* Wilcox (*10*). The weft of fungous hyphae produced by all of these fungi is white and ocupies the same tissues. It is obvious then that the disease caused by these fungi can not be separated solely on the basis of or the presence of mycelium and rhizomorphs—general symptoms of stump destruction and weak plant growth—when not black. While the *A. mellea* fungus has not been reported on dewberry, it attacks the blackberry (*5*) which is susceptible to the disease described here. It is certain that the only safe method of procedure

in determining the cause of root-rot (perhaps most appropriately stem-rot) diseases of berries, is by means of sporophores, the method by which the Agaricaceae are now classified. These fungi may live in the conductive tissues of roots and stems for many years before the production of sporophores takes place, but these can be obtained more quickly in pure culture without much difficulty, according to the present researches and to those of Gilmore (*3*), and Molisch (*7*).

The southern blackberry (*Rubus villosus* Ait.) and wild southern dewberry (*Rubus trivialis* Michx.) are both susceptible to the root-rot disease and further inoculations are being carried out to determine the relation of the fungus to other plants in the infected area.

SUMMARY

A root-rot disease of the Lucretia dewberry *Rubus procumbens* Muhl. var., *roribaccus* Bailey is causing severe damage throughout the sand hills from Sanford to Hamlet, N. C. It was reported in 1922, and appears to have become worse in recent years since plants have been plowed up in some fields because of being diseased and unprofitable, a condition which has not been described heretofore.

The symptoms of the disease are a stunted growth of the plant resulting from a diseased root system; wilting, caused by a recent attack of the disease on the canes below old spurs left after pruning, and by the death of the roots; girdling of canes; small and bushy purplish leaves; wefts of white fungous hyphae in bark, cambium, and rays, hyaline mycelium in xylem, and pith cells; white to tan and brown rhizomorphs on dead and live parts of roots and canes; and the decay of the wood, which becomes soft.

The disease is caused by a parasitic variety of *Collybia dryophila* Fr. which is widely distributed geographically in Europe and America, where it has been studied extensively, but the parasitic variety described here may not be widely distributed, since this is the first report of a parasitic variety of the *C. dryophila* "group."

The causal organism is isolated by plating on prune agar mycelium and rhizomorphs from recently infected live parts of the plant, since recently infected tissues are not apt to be infected with saprophytic fungi, which are difficult to eradicate and usually develop more quickly than the parasite. The fungus is difficult to isolate when neutral media are used, but grows well on plant coctions of potato, sweet potato, rice, apple and corn meal, on barnyard manure; and on Cook's synthetic medium, when large amounts of inoculum are transferred or when the media are made slightly acid.

The sporophores were obtained on dewberry medium only, but greater production was brought about by adding cooked prune extract to the medium. In pure culture the first sporophores were obtained in 30 days, while maximum production was obtained in from 60 to 90 days. The duration of sporophore production in this medium is six months.

The description of the parasitic variety varies only slightly from description given by others. The plant is solitary, gregarious, and subcaespitose; the pileus is white, tan, and shades of brown, and 2 to 8 centimeters broad; the stipe is striate, stuffed, and 3 to 15 centimeters long; the lamellae are some shade of white, narrow, crowded, and adnexed; the spores are hyaline, pip-shaped, and 4 to 8 μ.

The life cycle of the fungus in North Carolina is attained without uniform production of sporophores, since there were no sporophores produced in the field during 1926.

The parasitic nature of the fungus was thoroughly tested. A pure culture of the fungus was first obtained. Healthy plants were inoculated and after the disease symptoms were produced, the fungus was reisolated from the diseased areas. Furthermore, the fungus has lived on inoculated plants throughout a period of nine months, constantly forming new rhizomorphs, and attacking roots and stems.

The disease is disseminated by means of infected plants and on pruning shears.

The plants known to be susceptible to this disease are the southern blackberry (*Rubus villosus* Ait.), wild southern dewberry (*R. trivialis* Michx.), and Lucretia dewberry (*R. procumbens* Muhl. var. *roribaccus* Bailey).

The symptoms of root rots of other plants caused by *Armillaria mellea* (Vahl) Quel. and *Clitocybe parasitica* Wilcox are more or less similar to those produced by *Collybia dryophila*, from which it is obvious that identification of root-rot diseases made solely on the basis of the presence of rhizomorphs and wefts of fungous hyphae in the tissues without the sporophores, is apt to be misleading, since classification of the Agaricaceae rests upon the sporophore characters.

LITERATURE CITED

(1) Buller, A. H. R.
1924. researches on fungi. v. 3, illus. London, New York [etc.].

(2) Coker, W. C., and Beardslee, H. C.
1921. the collybias of north carolina. Jour. Elisha Mitchell Sci. Soc. 37: 83–107, illus.

(3) Gilmore, K. A.
1926. culture studies of psilocybe coprophila. Bot. Gaz. 81: 419–433, illus.

(4) Kauffman, C. H.
1918. the agaricaceae of michigan. Mich. Geol. and Biol. Survey Pub. (biol. ser. 5) 26, v. 1, illus.

(5) Lawrence, W. H.
1910. root diseases caused by armillaria mellea in the puget sound country. Wash. Agr. Expt. Sta. Bul. (spec. ser.) 3, 16 p., illus.

(6) McIlvaine, C., and Macadam, R. K.
[1902]. toadstools, mushrooms, fungi edible and poisonous. one thousand american fungi. Rev. ed., 729 p., illus. Indianapolis.

(7) Molisch, H.
1904. leuchtende pflanzen. eine physiologische studie. 168 p., illus. Jena.

(8) Peck, C. H.
1897. new york species of collybia. N. Y. State Mus. Ann. Rpt. 49 (1): 46–69.

(9) Schrenk, H. von, and Spaulding, P.
1909. diseases of deciduous forest trees. U. S. Dept. Agr., Bur. Plant Indus. Bul. 149, 85 p., illus.

(10) Wilcox, E. M.
1901. a rhizomorphic root rot of fruit trees. Okla. Agr. Expt. Sta. Bul. 49, 32 p., illus.

(11) Wolf, F. A., and Dodge, B. O.
1926. anthracnose of dewberries and its control. N. C. Agr. Expt. Sta. Bul. 248, 16 p., illus.

POMEGRANATE BLOTCH [1]

By FREDERICK A. WOLF

Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The pomegranate (*Punica granatum* L.) is not grown on a commercial scale in southeastern United States, but is extensively planted as an ornamental. On this account, no doubt, little attention has been given to its diseases. During the summer of 1925, however, the writer noted the occurrence on this plant, in several localities in Florida, of a disease which affects both the foliage and the fruits. An examination showed that this disease is identical with one which was first collected in 1909 in Texas and ascribed to *Cercospora lythracearum* H. & W.[2] Since these brief mycological notes appear to constitute the only published account of the fungus, an attempt was made to determine its life cycle and to gain additional information in regard to the disease. It has been found that the disease not only manifests itself by foliar lesions but also by characteristic blotches upon the fruit, so that it may properly be designated by the common name "blotch." In addition it has been found that the parasite which causes blotch possesses in sequence a conidial, a spermagonial, and an ascigerous stage. The observations to date will therefore be briefly summarized as a contribution to our knowledge of pomegranate diseases.

APPEARANCE OF LESIONS

The pomegranate in Florida retains some of its foliage throughout the entire year, so that affected leaves may be found at any season. The presence of circular or somewhat angular brown areas 4 to 5 mm. in diameter characterize the leaf-spot form of this disease. (Fig. 1, D, and pl. 1, A.) The margins of the lesions when viewed with the aid of a hand lens by transmitted light are pink and somewhat translucent. Affected leaves are pale green, and usually there are fewer than 20 spots per leaf. Diseased leaves fall prematurely in sufficient quantity to keep the ground covered. During periods of high relative humidity the lower surface of lesions is covered with dense aggregates of conidiophores and conidia which en masse appear brown. The conidial fructifications appear on the upper surface less commonly and in less abundance.

The first evidences of disease on the fruit are small but conspicuous dark-brown spots. These are circular in outline but by unequal radial growth soon become irregular blotches. By coalescence of these blotches a considerable proportion of the surface of the fruit becomes discolored. (Fig. 1, E, and pl. 1, B.) No pitting nor fissuring

[1] Received for publication May 20, 1927; issued October, 1927.

[2] HEALD, F. D., and WOLF, F. A. NEW SPECIES OF TEXAS FUNGI. Mycologia 3: 5-22. 1911.
——— and WOLF, F. A. A PLANT DISEASE SURVEY IN THE VICINITY OF SAN ANTONIO, TEXAS. U. S. Dept. Agr., Bur. Plant Indus. Bul. 236, 129 p., illus. 1912.

of these affected tissues occurs. When affected fruits are provided with suitable moisture conditions, such as are present when they are placed in a moist chamber, the surface of the lesions becomes covered with a velvety brown layer of conidiophores and conidia.

THE CAUSAL FUNGUS

The Cercospora or conidial stage of the fungus which causes these lesions may be found on the foliage at any time throughout the year. The conidiophores occur in dense aggregates most commonly on the

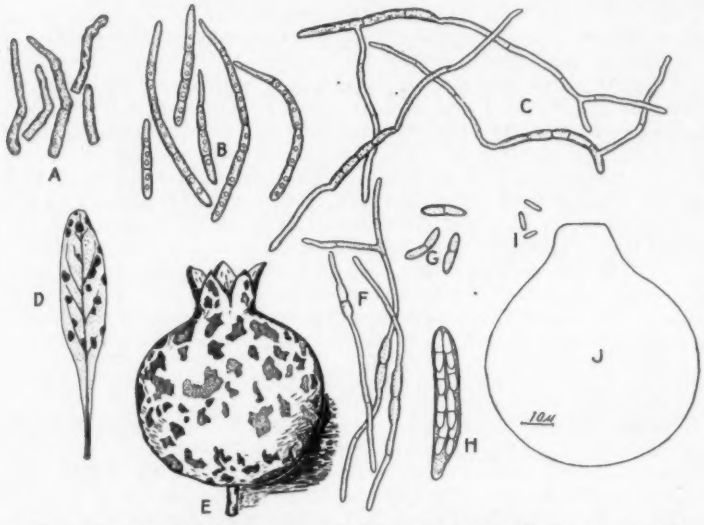
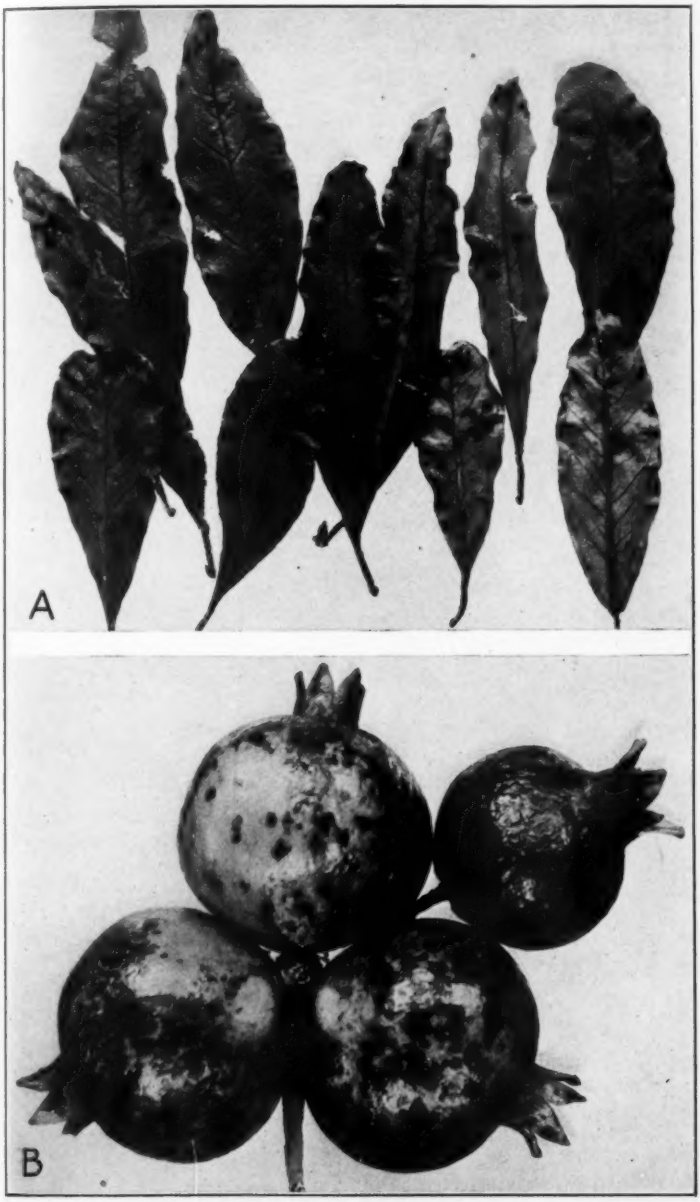


FIG. 1.—Pomegranate blotch and its causative fungus, *Mycosphaerella lythracearum*: A, conidiophores of the blotch fungus; B, conidia; C, germination of conidia; D, distribution of lesions on pomegranate leaf; E, distribution of lesions on fruit; F, germinating ascospores; G, ascospores; H, ascus; I, spermatia; J, perithecium in outline. (All except D and E drawn to scale shown in J)

lower leaf surface. They measure 20μ to 30μ by 3μ to 4μ and are faintly brown. (Fig. 1, A.) The conidia are filiform, clavate, subhyaline 2 to 5 septate, and measure 30μ to 56μ by 3μ to 3.5μ. (Fig. 1, B.) They germinate readily in water or on solid media with the formation of several germ tubes. (Fig. 1, C.) The growth of the colonies is rather slow on potato-dextrose agar, since about a month is required for the production of a colony 1 cm. in diameter. The surface of the colonies in contact with the medium is olivaceous in color and the exposed surface smoky. The mycelium is densely compacted except at the exposed surface.

Diseased leaves which were collected during August and placed in a sheltered situation in contact with the ground, when examined during October, were found to contain numerous globular black bodies in the spots which had been killed by the Cercospora. Free-hand sections showed that these bodies were pycnidial in structure,



A.—The leaf-spot form of pomegranate blotch
B.—Blotch lesions on pomegranate fruits

that they opened to the lower leaf surface, and that they were filled with numerous hyaline rodlike pycnospores measuring 3μ to 5μ by 1μ to 1.5μ. All efforts to germinate these pycnospores have resulted in failure, which fact is regarded as evidence that they are not the conidia of a saprophytic species of Phyllosticta but are homologous with what have been termed spermatia. Their small size, their occurrence only in the tissues killed by Cercospora and not in the tissues surrounding the lesions, the time of their formation, which corresponds with that of the appearance of spermagonia of other ascomycetous fungi, and the fact that they are succeeded in due time by the perithecial stage, are regarded as additional evidence that these pycnospores are spermatia.

At the time when the spermagonia were abundantly present, stromata were noted to be forming within the palisade parenchyma. These stromata proved from subsequent observation to be the initials of perithecia. Mature perithecia, however, were not observed until March 23. They were then present in abundance and occupied the lesions produced by the Cercospora stage and appeared as tiny black elevations projecting from the upper leaf surface. They are globular in outline, vary in diameter from 75 to 95 μ, and open to the surface by means of a short blunt papilla. (Fig. 1, J.) The wall is composed of a thin layer of brown cells.

The asci are eight-spored, cylindrical, without paraphyses, and cling together in a cluster when crushed out of the perithecium. They measure 42μ to 50μ by 6.5μ to 8μ. (Fig. 1, H.) The ascospores are biseriate, unequally two-celled, hyaline, curved, and measure 11μ to 14μ by 2.7μ to 3.5μ. (Fg. 1, G.)

Isolations from ascospores were obtained by inverting poured potato-dextrose agar plates above leaves bearing mature perithecia. The ascospores are thus forcibly expelled and lodge on the surface of the medium. Germination occurs within 18 hours by the emergence of a germ tube from each end of the ascospore. (Fig. 1, F.) The mycelial growth is rather slow and is at first olivaceous.

Parallel cultures isolated from conidia and from ascospores were made on carrot agar, Czapek's agar, Dox's agar, prune agar, glycerine agar, cornmeal agar, potato-dextrose agar, apple agar, peach agar, sterilized pigeon-pea stems, potato cylinders, and sterilized leaves, twigs, and fruits of pomegranate. All cultures on the same medium were entirely similar in growth characters. Further, all cultures both from ascospores and from conidia have remained sterile, so that it has been impossible to secure evidence of the genetic relationship of conidial and ascogenous stages in the usual manner. This failure to form conidia in culture has precluded the possibility of making inoculations from pure cultures as a means of showing the relationship of the two stages and at the same time of proving the pathogenicity of this fungus. However, there is ample reason to believe that the two stages are related, because (1) the colonies from conidia and from ascospores are indistinguishable; (2) conidia, spermagonia, and the perithecial initials are present at the same time in autumn in the same lesions; (3) the perithecia are present in abundance in spring in the areas resultant from infection by the Cercospora stage; (4) they do not occur in the tissues surrounding the lesions produced by Cercospora, and no other ascomycete is associated with the diseased areas; and (5) these observations accord with well-known facts, since

other species of Cercospora are known to possess an ascigerous stage of the type described for the pomegranate fungus. Further, field observations leave no doubt that the Cercospora stage is virulently parasitic.

SYSTEMATIC DESCRIPTION

The characters presented by this fungus are manifestly those of the genus Mycosphaerella as employed by Johanson,[3] to which genus it is assigned. Apparently no ascomycete of this genus has been described on *Punica granatum*, and it is not evidently like any of the Mycosphaerellas on Lythraceae or closely related plants. Furthermore, it is not believed to be advisable solely on the basis of morphological similarity to assign it to any of the numerous species of Mycosphaerella which have been described on other hosts. Since the Mycosphaerella under consideration is associated with pomegranate, and since it is highly probable that it is connected with *Cercospora lythracearum*, it may appropriately be given the name *Mycosphaerella lythracearum* with the following diagnosis:

Mycosphaerella lythracearum n. sp.[4] Syn. *Cercospora lythracearum* Heald and Wolf.

Ascigerous stage.—Perithecia black, epiphyllous, aggregated on spots killed by conidial stage, globose, 75μ to 95μ in diameter, immersed with papilla protruding through the epidermis. Asci cylindrical, clustered, aparaphysate, 42μ to 50μ by 6.5μ to 8μ; spores biseriate, slightly curved, colorless, unequally once septate, 11μ to 14μ by 2.7μ to 3.5μ. In fallen leaves of *Punica granatum*.

Conidial stage.—Lesions on leaves angular to circular, brown, 4 to 5 mm. in diameter; on fruit, irregular dark blotches. Dense aggregates of conidiophores 20μ to 30μ by 3μ, subhyaline, amphigenous, mostly hypophyllous. Conidia filiform, clavate, 30μ to 56μ by 3μ to 3.5μ, hyaline, 2 to 5 septate. Parasitic on leaves and fruits of *Punica granatum*.

Spermagonial stage.—In autumn epiphyllous spermagonia appear in lesions produced by the Cercospora stage. Spermatia 3μ to 5μ by 1μ to 1.5μ.

Available records of collections indicate the occurrence of pomegranate blotch in several localities in Texas, Florida, Alabama, and Bermuda.[5]

LATIN DESCRIPTION

Peritheciis atris, epiphyllis, gregariis, globosis, epidermide velatis poro pertusis, 75–95μ; ascis cylindricis, fasciculatis, aparaphysatis, 42μ to 50μ by 6.5μ to 8μ; sporidiis distichis, leviter curvulis, hyalinis, bicellularibus, loculis subaequalibus, 11μ to 14μ by 2.7μ to 3.5μ. Hab. in foliis dejectis Punicae granati. Status conidicus: maculis in foliis angularis vel rotundatis, brunneis, 4 to 5 mm. in diam.; laesiones in fructis irregularibus, atris; conidiophoris dense aggregatis, 20μ to 30μ by 3μ, dilute brunneis; conidiis filiformibus, clavatis, 30μ to 56μ by 3μ to 3.5μ, hyalinis, 2 to 5 septatis; spermatiis autumno in spermagoniis, 3μ to 5μ by 1μ to 1.5μ hypophyllis. Hab. in foliis fructisque vivis Punicae granati.

Specimens have been made available by deposition in the collections of the Office of Mycology and Disease Survey, Bureau of Plant Industry, Washington, D. C.

SUMMARY

This report concerns a disease which appears on the foliage and fruits of *Punica granatum*. It has hitherto been known as a leaf spot but manifests itself also by characteristic blotches on the fruit. It is known to occur in Texas, Florida, Alabama, and Bermuda.

[3] JOHANSON, C. J. SVAMPAR FRÅN ISLAND. Öfvers. K. [Svenska] Vetensk. Akad. Förhandl. 41 (9): 163, 1884.

[4] There are mycologists who would use the generic name Sphaerella in the sense employed in the following: SACCARDO, P. A. SYLLOGE FUNGORUM. v. 22, p. 120. Patavii. 1913.

[5] Collections from Alabama were made by H. R. Fulton and from Bermuda by H. H Whetzel and L. Ogilvie.

Pomegranate blotch is caused by an ascomycetous fungus herein given the name *Mycosphaerella lythracearum* n. sp. The conidial stage of this fungus, *Cercospora lythracearum*, is present throughout the entire year in Florida on green foliage.

A spermagonial stage occurs during autumn on the lower surface of lesions caused by the Cercospora stage.

Mature perithecia are present in early spring on fallen leaves.

The relationship of conidial and perithecial stages is evidenced (1) by their occurrence in due time in the same lesions, (2) by the similarity of cultures from conidia and ascospores, and (3) by the fact that a number of Cercosporas are known to possess a Mycosphaerella stage.

A SUBCUTANEOUS TUMOR IN A PRIMATE CAUSED BY TAPEWORM LARVAE EXPERIMENTALLY REARED TO MATURITY IN DOGS[1]

By BENJAMIN SCHWARTZ

Associate Zoologist, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

This paper contains a description and discussion of a tapeworm of which the larval stage, known to zoologists as a coenurus, was found causing a large subcutaneous tumor in a primate (*Theropithecus obscurus*), and from which the adult stage, a strobilate tapeworm, was experimentally reared in dogs. Despite the fact that these bladder worms and experimentally reared tapeworms as investigated by the writer appear to be morphologically indistinguishable from one of the known dog tapeworms (*Multiceps serialis*), the former are regarded in this paper as representing a variety of that species. The reasons for differentiating these worms from *Multiceps serialis* are based on experiments involving the intermediate host relationship of the parasite studied by the writer. The latter form will be referred to in this paper as *Multiceps serialis* var. *theropitheci*. This designation conforms to a certain tendency among parasitologists to designate varieties of parasites by the generic name of the host or by a derivative of that name.

Parasites of primates have considerable potential significance in human medicine, as Stiles and Orleman (*10*)[2] have recently noted, and this fact warrants a detailed study of this coenurus as possibly one that is capable of developing in man with distinctly unpleasant consequences. As a matter of fact two very closely related larval tapeworms, *Multiceps multiceps* and *Multiceps glomeratus*, have been reported from man, the former from one of the lateral ventricles of the brain and the latter from one of the intercostal muscles.

The parasites were collected by Robert Formad, of the pathological division of the Bureau of Animal Industry, in the course of a necropsy on a baboon (*Theropithecus obscurus*) from the National Zoological Park at Washington, D. C. The baboon was killed early in April, 1925, because it was deformed by a large subcutaneous tumor in the right thoracic region which rendered the animal unsuitable for exhibition purposes. Formad removed the tumor, recognized it as parasitic in nature, and referred it to the zoological division for identification. The following is a part of a report submitted by Formad in regard to the post-mortem appearance of the tumor:

> Carcass in good condition. Large cystic tumor, parasitic in nature and honeycombed in appearance, was situated externally on the right thoracic region, adhering to the skin around the nipples. A fistulous and necrotic tract extended for about 1 inch into the tumor.

[1] Received for publication Apr. 26, 1927; issued October, 1927.
[2] Reference is made by number (italic) to "Literature cited" p. 480.

DESCRIPTION OF TUMOR

In its unpreserved state the tumor weighed about 1,500 gm. The preserved specimen is more or less spherical in shape and somewhat asymmetrical, being about 20 cm. long by about 16 cm. wide. Its membraneous surface presents a number of openings of which some are more or less circular, others irregular in shape. The openings lead into spaces which communicate with adjoining spaces through openings, the entire tumor presenting a honeycombed appearance and the intercommunicating spaces being more or less completely filled with bladder worms (larval tapeworms).

About one-half of the membranous surface of the cyst appears wrinkled and folded, with numerous bladder worms protruding through the openings. The remaining portion of the external surface of the cyst is fairly smooth and contains but few openings. The general shape and appearance of the tumor are shown in Plate 1, in which a number of the larger openings on the wrinkled surface of the cyst wall are clearly visible.

DESCRIPTION OF BLADDER WORMS

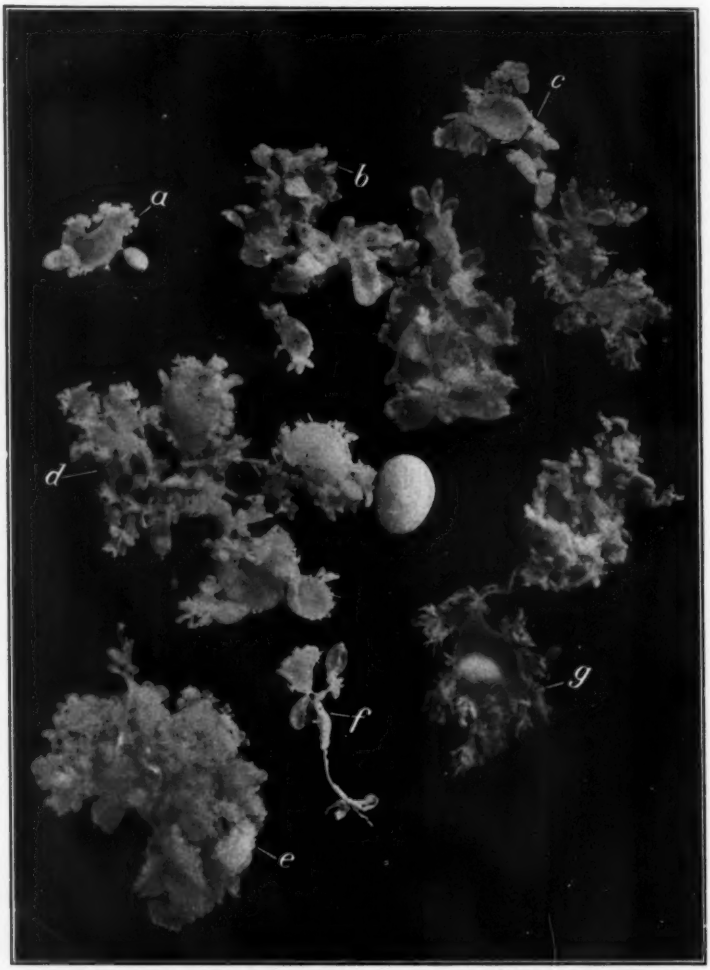
Many of the bladder worms are more or less elliptical, some are spherical, and others are irregular in shape. They occur in clusters, the individuals containing, as a rule, several heads. The bladder worms show considerable variation in size and shape and in external modification, most of them containing a variable number of fringed structures on the surface. Some appear to be perfectly smooth, however, and occasionally occur as single forms with a small stalk or pedicle by which they were probably at one time attached to a cluster. Although the bladder worms are commonly found in grape-like clusters, some forms exhibit a branching structure, being attached to the parent stem by short stalks. The appearance of the colonial bladder worms and their union to one another as well as their mode of branching are shown in Plate 2 and in Figure 1, in which various shapes of individual bladder worms, cluster formation, and the attachment of bladder worms to stalks by means of short stems are clearly illustrated.

FIG. 1.—A cluster of bladder worms showing the attachment of the coenuri to the cluster by means of stems. About natural size

Although a number of sterile bladder worms were observed, as well as a number which contained but a single head each, the majority of the mature individuals contained several heads each. Figure 2 shows a cluster containing two large and several smaller bladder worms. The large bladder worms contain several heads each, most of the heads being evaginated. Figure 3, *a*, shows a bladder worm with eight invaginated and one evaginated head, and Figure 3, *b*, shows a bladder worm with seven invaginated heads and a smaller bladder worm attached to it with three invaginated heads.

The question of the specific identity of the coenurus stage of the tapeworm genus Multiceps involves primarily the number, size, and shape of the hooks and incidentally the other head structures, notably the suckers and rostellum. Examination of the bladder-worm heads obtained from the tumor showed them to be densely

Tumor showing openings on membranous surface. The irregularly shaped, whitish objects are the protruding bladder worms



A group of bladder worms, *Multiceps serialis* var. *theropitheci*. Natural size. *a*, A small group of bladder worms, the one on the right being attached by means of a small stem; *b*, *c*, and *d*, groups of bladder worms showing variation in size and shape of the individual coenuri; *e*, a grapelike cluster of bladder worms; *f*, a group of bladder worms that exhibit a dendritic mode of branching; *g*, two clusters of bladder worms connected by a strand of tissue

crowded with calcareous corpuscles. In unpressed preparations the diameter of the scolex or head is about 774μ; the diameter of the suckers ranges from 235μ to 260μ. There is a double crown of from 28 to 32 hooks. In one specimen the large hooks range in length from 135μ to 144μ and the small hooks from 90μ to 95μ. In another specimen the following measurements of the individual large hooks were made: 144μ, 135μ, 135μ, 139μ; and in the same specimen the following measurements of the lengths of the small hooks were made: 95μ, 90μ, 81μ, 95μ, 90μ, 95μ. In still another specimen four large hooks were 135μ long, one was 138μ, another 140μ, another 144μ, and one 148μ. Small hooks in the same specimen showed the following lengths: Two, 90μ; three, 95μ; two, 99μ; one, 108μ; and one, 118μ. The extremes are 135μ to 148μ for large hooks and 81μ to 118μ for small hooks.

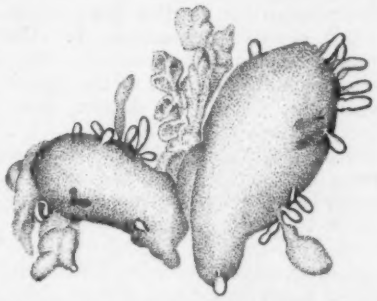


FIG. 2.—A cluster consisting of two large and several smaller bladder worms, most of the heads being evaginated. Enlarged

The large hooks (fig. 4) have a blade of moderate curvature, a short thick handle, sinuous in outline and distinctly marked off at the point of union with the blade by a curvature, the convexity of the curve being ventral. The guard is short, thick, and bluntly rounded. The small hooks (fig. 4) have a blade of from moderate to strong curvature, a short, thick, blunt handle, and a guard resembling that of the large hooks.

FIG. 3.—Bladder worms with evaginated heads. Enlarged

FIG. 4.—Large and small hooks of larval worms, *Multiceps serialis* var. *theropitheci*

KNOWN SPECIES OF MULTICEPS

In recent years Railliet and Mouquet (7) and Joyeux (3) have summarized the known species of Multiceps, a total of 12 species being referred to that genus as follows: (1) *M. multiceps* (Leske, 1780), the intermediate (coenurus) stage of which occurs in the brain and spinal cord of sheep and other herbivores and the adult stage in dogs. Records of the occurrence of the larval stage of this tapeworm

in a hog and in man have also been published. (2) *M. serialis* (P. Gervais, 1847), the larval form occurring in subcutaneous and in intermuscular tissue of various Leporidae and other rodents, the adult stage in dogs. (3) *M. lemuris* (Cobbold, 1861), the larval form occurring in the liver, lungs, and pleura of *Lemur catta*, the adult stage being unknown. The host of this parasite has been referred to as *Lemur macaco*. Railliet and Mouquet (*7*) have shown that the specific name of the host is *L. catta*. (4) *M. polytuberculosus* (Mégnin, 1879), the larval form occurring in a rodent (*Jaculus jaculus*) according to Railliet and Mouquet (*7*). (5) *M. brauni* (Setti, 1897), the adult stage occurring in the dog, and the larval form occurring under the skin of a mouse (*Gerbillus pyramidum*), according to von Linstow (*5*). (6) *M. glomeratus* Railliet and Henry, 1915, the larval form occurring in the abdominal cavity of a mouse *Gerbillus hirtipes*), the adult stage being unknown. (7) *M. spalacis* (Diesing, 1863), the larval form occurring in *Georhychus capensis*, the adult form being unknown. (8) *M. gaigeri* Hall, 1916, the larval form occurring under the skin, in the intermuscular tissue on the viscera, and in the central nervous system of the goat (*Capra hircus*), the adult stage occurring in the dog. (9) *M. ramosus* Railliet and Marullaz, 1919, the larval form occurring in subcutaneous tissue of the perineum of *Cynomolgus* (*Macsacu*) *sinicus*, the adult stage being unknown. (10) A somewhat doubtful form, *M.* (?) *ovis tragelaphi* (Cobbold, 1861), the larval form occurring in *Ovis tragelaphus* and, according to Meggitt (*6*), in *Ammotragus lervia*, the adult being unknown. (11) *M. clavifer* Railliet and Mouquet, 1919, the larval form occurring in a rodent (*Myopotamus coypus*), the adult stage being unknown. To the above species must be added (12) *Multiceps radians* Joyeux, Richet, and Schulmann 1922 (*4*), the larval form occurring in a white mouse, the adult stage being unknown.

As regards the size of the hooks, the coenurus from the baboon which is described in this paper corresponds to *Coenurus* (*Multiceps*) *serialis*. The only other species to which it may possibly correspond on the basis of the size and number of hooks is *Multiceps brauni*. The latter species has large hooks ranging from 130μ to 140μ long, occasionally only 95μ to 100μ long, and small hooks ranging from 85μ to 90μ, occasionally only 70μ to 75μ long, these measurements being based on the adult stage. Both the large and the small hooks of the specimen from the baboon exceed these measurements. According to Hall (*2*), the guard of the large and small hooks of *M. brauni* tends to be bifid, a character not observed in the present writer's specimens from the baboon. So far as concerns the morphology of the hooks, which constitutes the best character on which to base a determination of a larval taenioid tapeworm, the specimens from *Theropithecus obscurus* appears to agree with those of *Multiceps serialis*.

EXPERIMENTAL TRANSMISSION OF THE COENURUS FROM THEROPITHECUS TO DOGS

In order to determine the adult stage of the tapeworm in question, feeding experiments were performed and the results noted in each case. These results, briefly summarized in a previous paper (*8*), follow:

EXPERIMENT 1

On April 8, 1925, dog 868 was fed one large cyst containing several heads. A fecal examination of this dog prior to feeding the bladder worm yielded the following results: Ova of Toxascaris, Dipylidium, and Trichuris present. The dog died April 29, 21 days after the cyst had been fed. The following parasites were removed from the dog at necropsy: *Toxascaris limbata*, *Trichuris vulpis*, *Dipylidium caninum*, and four small taenioid tapeworms (Multiceps). The total length of each tapeworm before fixation was ascertained to be 9.2 cm., 7.7 cm., 4.4 cm., and 2.3 cm., respectively. The head characters of these tapeworms were identical with those of the larval forms which were fed to the dog. The following are the detailed measurements of one specimen: Total length after fixation, 7.2 cm.; total number of hooks, 32; diameter of head, 732μ; diameter of rostellum, 270μ; large hooks, 144μ to 149μ long; small hooks, 90μ to 95μ long. A short distance below the neck the segments are 72μ long by 666μ wide; from this point the segments gradually increase in length to 144μ without any appreciable increase in width. Segments 306μ to 324μ long are about 864μ wide; segments 918μ long are 1.2 mm. wide and show the presence of testes.

EXPERIMENT 2

Dog 890 was fed several bladder worms on April 8, 1925, containing a total of about 15 heads. This dog was not examined for parasites before feeding, but several days after feeding an examination was made and the following parasite ova were found: Toxascaris, Dipylidium, and Taenia. The dog was killed on June 6, 1925, approximately two months after experimental feeding. In addition to *Toxascaris limbata*, *Dipylidium caninum*, and *Taenia pisiformis*, the dog contained about a dozen specimens of Multiceps. Two specimens of the last-named species were examined and the total number of hooks found were 30 and 32, respectively. The large hooks were 145μ to 153μ long; the small hooks from 90μ to 95μ. The suckers are from 235μ to 252μ in diameter.

EXPERIMENT 3

Dog 918 was fed several bladder worms on April 11, 1925. Fecal examination of this dog prior to feeding showed the presence of numerous Belascaris ova. On May 25, approximately six weeks after experimental feeding, several tapeworm proglottids and one head were found in the feces of this dog. The hooks corresponded in size and number to those of the bladder worms fed. On June 1, 1925, about seven weeks after experimental feeding, proglottids containing eggs were found in the feces of this dog. Two days later additional proglottids containing eggs were found in the feces. This dog died on June 14, 1925, but was accidentally disposed of without being examined.

EXPERIMENT 4

Dog 919 was fed several bladder worms on April 11, 1925. A fecal examination two days later showed the presence of Ancylostoma and Trichuris ova. On May 8, 1925, approximately four weeks after feeding, the dog was killed. Post-mortem examination revealed the presence of the following parasites: *Trichuris vulpis*, *Dipylidium caninum*, *Anchylostoma caninum*, and 15 taenioid tapeworms (Multi-

ceps) with heads. One of the tapeworms was found in the duodenum, the remaining specimens being found in the jejunum and ileum, the latter portion of the intestine containing fewer tapeworms than the former. Several of the tapeworm heads from this dog were examined and they corresponded morphologically to those recovered in the previous feeding experiments described above.

EXPERIMENT 5

On April 7, 1925, several bladder worms each containing two or more heads were fed to a cat. This animal was kept under observation for more than two months during which period its feces was examined from time to time for proglottids and for ova but with negative results.

MORPHOLOGY OF TAPEWORMS EXPERIMENTALLY REARED IN DOGS AS A RESULT OF FEEDING THEM COENURI FROM THEROPITHECUS

The head of the tapeworm is more or less quadrangular when viewed en face (fig. 5), its diameter varying from about 738μ to slightly over 1 mm. The rostellum is from 270μ to about 360μ in diameter, bearing a double crown of from 28 to 32 hooks. The large hooks (fig. 6, A and D) are from 144μ to 153μ long. The blade is of moderate curvature; the handle is somewhat sinuous in outline, rather short, and of varying shape and diameter. In some of the large hooks the handle is slightly tapering, in others it ends bluntly. The handle of practically all the large hooks that were examined showed a tendency to turn dorsally at the distal extremity. The guard is short, its distal extremity being blunt in some specimens and somewhat tapering in others. The small hooks (fig. 6, B and C) are from 90μ to 103μ long. The blade is of moderate curvature. The handle is short and thick. In some specimens the handle shows a slight curvature, the convexity of the curve being ventral; in other specimens this curvature appears to be absent. The guard is short and thick, its distal extremity being more or less pointed. The suckers are about 270μ in diameter. The entire strobila is from 29 to 48 cm. long. The neck is very distinct. The mature segments (figs. 7 and 8) are wider than long, about 2 mm. long by about 3 mm. wide. The gravid segments are longer than wide, usually 7 or 8 mm. long, occasionally about 10 mm. long by 3 to 4 mm. wide. The entire strobila

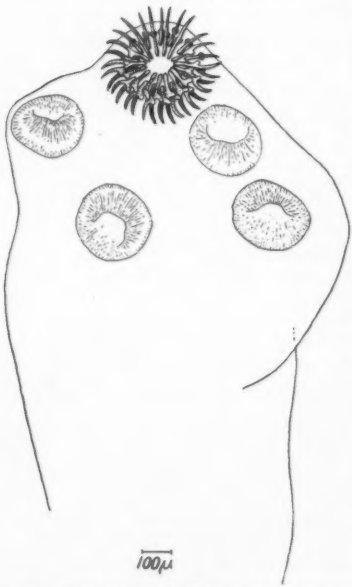


FIG. 5.—Head of strobilate tapeworm, *Multiceps serialis* var. *theropitheci*

is very dense and opaque. The genital pores are irregularly alternate. The genital papilla is very prominent, being situated in the middle of, or, more usually, somewhat posterior to the middle of the segment.

MALE GENITALIA

In immature specimens the testes do not extend to the field of the median stem of the uterus, but in fully mature specimens the testes spread to this field (figs. 7 and 8) and extend close to the margin of the vagina and the vas deferens. The testes also extend to the margin of the ovaries and posteriorly beyond the ovaries to the vitellarium, but not behind the vitellarium. The vas deferens originates at some distance from the median uterine stem on the pore side of the proglottid. The cirrus pouch is transversely elongated and narrow.

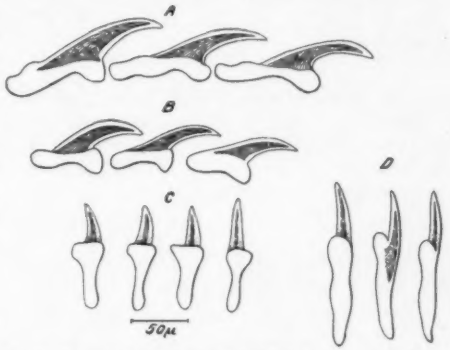


FIG. 6.—Large and small hooks of strobilate tapeworm, *Multiceps serialis* var. *theropitheci*. Enlarged

FEMALE GENITALIA

In some segments the ovaries are only slightly dissimilar in size; in other specimens there is a marked difference in size between the two ovaries. The vitellarium is elongated along the transverse axis of the segment. In some specimens it does not extend laterally as far as the margins of the ovary; in other specimens it reaches the margins. In some segments (fig. 8) the vagina

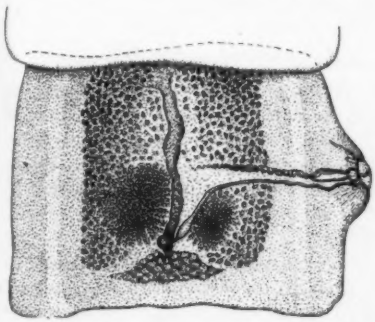


FIG. 7.—Sexually mature segment of *Multiceps serialis* var. *theropitheci*. Enlarged

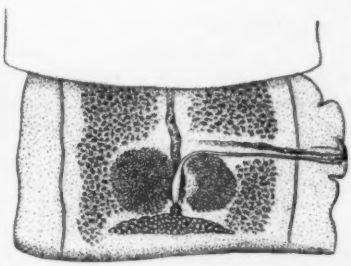


FIG. 8.—Sexually mature segment of *Multiceps serialis* var. *theropitheci*. Enlarged

shows no loop and is practically straight; in other segments (fig. 7) it is somewhat sinuous in the region of the excretory canal, and a more marked loop than that shown in Figure 7 was observed in a number of segments. The gravid segments show a median uterine stem with numerous branches and subbranches, making an actual count difficult. The eggs are variable in size, ranging from 25μ to 42μ in diameter.

The morphology of the adult tapeworm corresponds closely to Hall's (*2*) description of *Multiceps serialis*. From the description of *M. serialis* as given by Hall and from an actual study of specimens of that species, no constant characters have been found that would serve to differentiate this worm from the form from the baboon, which was experimentally reared in four dogs.

EXPERIMENTS ON THE ATTEMPTED TRANSMISSION OF THE HEXACANTH EMBRYOS TO VARIOUS EXPERIMENTAL ANIMALS

In order to obtain additional evidence that would throw light on the specific identity of this tapeworm, the following experiments were performed:

EXPERIMENT 6

Two gravid proglottids from dog 918 were fed to each of two rabbits on June 1, 1925. One rabbit died on July 6, 1925. Post-mortem examination showed no evidence of infestation with larval tapeworms. The second rabbit was killed a month later, and the results were likewise negative.

EXPERIMENT 7

On June 4, 1925, a gravid segment from dog 918 was fed to a guinea pig which was killed within two months. The results were negative.

EXPERIMENT 8

A ripe proglottid from dog 918 was chopped up and fed on bread to three white mice. The mice were killed six weeks later and showed no evidence of infestation with larval tapeworms.

EXPERIMENT 9

On June 13, 1925, one gravid segment from dog 918 was fed to each of two guinea pigs and one gravid segment from the same dog was fed to one rabbit. The guinea pigs were killed two months later and the rabbit six months later. The results were negative.

EXPERIMENT 10

On June 24, 1925, each of two rabbits was fed one gravid segment from dog 890 and each of three guinea pigs was fed one-half gravid segment from the same dog. Chopped up gravid proglottids from this dog were also fed on bread on the same day to six rats. These animals were kept for about six months. Post-mortem examinations were negative.

EXPERIMENT 11

Two gravid segments from dog 890 were fed to each of two lambs on June 23, 1925. One lamb was accidentally disposed of so that no post-mortem examination was made. The second lamb was killed about five months after feeding and the results were negative.

DISCUSSION

The failure to rear the larval stage of this tapeworm in rabbits throws some doubt on the biological identity of the worm with *Multiceps serialis*, since the rabbit is the normal intermediate host of this species of tapeworm. While the worm from Theropithecus is morphologically related to, if not identical with, *M. serialis* it

appears to be biologically distinct, as far as can be judged by the results of feeding experiments described in this paper. While admitting the soundness of the proposition that zoological species should be based on morphological characters, it is important in the case of parasites to take cognizance of biological differences as evidenced by host relationship, and on the ground of an apparent biological distinction between *M. serialis* and the form discussed in this paper, the writer is inclined to regard the species from Theropithecus experimentally reared in dogs as a variety of *M. serialis*, for which the name *Multiceps serialis* var. *theropitheci* is proposed. Assuming that the two forms are morphologically identical, it may be suggested as an interesting speculation that in adapting themselves to new and unusual hosts parasites may undergo profound changes in their physiological organization without any apparent corresponding morphological modifications, and that such physiological species probably constitute the starting point which may ultimately lead to a definite morphological deviation from the parent species. An apparent physiological differentiation appears to have occurred in genera of parasitic mites such as Sarcoptes, Psoroptes, Chorioptes, and Notoedres, as some species of these genera from different hosts appear to be morphologically identical but are apparently more or less restricted to their host species. Among nematodes the case of *Ascaris lumbricoides* is of special interest in this connection. Although the human and swine Ascaris appear to be morphologically indistinguishable, considerable experimental and epidemiological evidence has been accumulated in recent years which shows that the form from the human host does not reach fertile maturity in the pig and vice versa. A similar case is that of the hookworm *Necator americanus* which occurs in human beings with a very similar form occurring in the pig, those from the latter host having been described as *N. suillus*. The two forms are very closely related and morphologically very similar. Attempts on the part of various workers to transmit experimentally *N. americanus* of human origin to pigs have invariably yielded negative results.

A similar host parasite relationship appears to hold true in certain plant parasitic nematodes, and this subject has been recently summarized by Steiner (*9*) as follows:

> A plant-parasitic nema apparently prefers always the host species or even the host variety on which its parents lived; the host principle, as established by Hopkins for insects, is also applicable to our plant-parasitic nemas.
>
> This preference for a certain host grows with the number of generations a nema population lives on it, the latter getting more and more specialized on this very host. This specialization may reach such a high degree that finally even new hosts of the closest taxonomical, physiological and chemical relationship to the old host are attacked no more or very lightly.

In the case of the species discussed in this paper, *Multiceps serialis* var. *theropitheci*, the host relationship is one that involves the larval stage only, the adult host being the same as that of *M. serialis*. The apparent intermediate host specificity of *M. serialis* var. *theropitheci* is correlated in this particular case with a larger cyst which has a more complex mode of branching than has been previously observed for *M. serialis*.

SUMMARY

A large subcutaneous cyst from a baboon (*Theropithecus obscurus*) is described in this paper as due to larval cestodes belonging to the genus Multiceps. A comparison of the hooks and of other morphological characters of this species of Multiceps with the other known species of the genus shows that the form from Theropithecus is closely related to, if not identical with, *Multiceps serialis*.

As a result of feeding one or more bladder worms (coenuri) to each of four dogs, the adult tapeworm was experimentally reared in these animals and its morphological organization was found to be similar to, if not identical with, that of *Multiceps serialis*. Owing, however, to a failure to develop the larval stage of the tapeworm in rabbits as a result of feeding them gravid proglottids of the tapeworms experimentally reared in dogs, the specific identity of the worm is open to question and in order to avoid confusion it is given varietal rank, the name *Multiceps serialis* var. *theropitheci* being proposed for it.

It is suggested that *Multiceps serialis* var. *theropitheci* probably represents a physiological species that has become adapted to a new intermediate host. The possibility that such host adaptations may ultimately lead to species differentiation among parasites appears to be indicated as a possibility.

LITERATURE CITED

(1) Diesing, K. M.
1863. revision der cephalocotyleen. abtheilung: paramecocotyleen. Sitzber. K. Akad. Wiss. Wien, Math. Naturw. Kl. 48: 200–345.

(2) Hall, M. C.
1919. the adult taenioid cestodes of dogs and cats, and of related carnivores in north america. U. S. Natl. Mus. Proc. 55: 1–94, illus.

(3) Joyeux, C.
1923. note sur le multiceps spalacis (moniez, 1880). Ann. Parasitol. Humaine et Compar. 1: 65–69, illus.

(4) ——— Richet, C., fils, and Schulmann, E.
1922. description d'un cénure trouvé chez la souris blanche de laboratoire. Bul. Soc. Zool. France 47: 181–186, illus.

(5) Linstow, [O.] von
1902. eine neue cysticercus-form, cysticercus taeniae brauni setti. Centbl. Bakt. [etc.] (I) 32: 882–886, illus.

(6) Meggitt, F. J.
1924. the cestodes of mammals. 282 p. London.

(7) Railliet, A., and Mouquet, A.
1919. cénure du coypou. Bul. Soc. Cent. Méd. Vét. 72: 204–211, illus.

(8) Schwartz, B.
1926. a subcutaneous tumor in a baboon due to multiceps larvae. (Abstract) Jour. Parasitol. 12: 159–160.

(9) Steiner, G.
1925. the problem of host selection and host specialization of certain plant-infesting nemas and its application in the study of nemic pests. Phytopathology 15: [499]–534, illus.

(10) Stiles, C. W., and Orleman, M. B.
1927. the nomenclature for man, the chimpanzee, the orang-utang, and the barbary ape. U. S. Pub. Health Serv., Hyg. Lab. Bul. 145, 66 p., illus.

○